On the Ecology and Restoration of

Podocarpus cunninghamii

in the Eastern South Island High Country

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Alwyn Williams

School of Forestry
University of Canterbury
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Abstract

Podocarpus cunninghamii is an endemic New Zealand conifer that, in pre-human times, formed extensive forest communities across the eastern South Island high country. Anthropogenic disturbances have reduced the distribution of *Podocarpus cunninghamii* communities such that they now exist mainly as small and isolated remnants within a highly modified, predominantly pastoral landscape. Very little is known of the ecology of high country *Podocarpus cunninghamii* communities, and without this information it is not possible to develop an ecological basis for their restoration. This thesis explores the ecology of *Podocarpus cunninghamii* in the eastern South Island high country, investigating factors that potentially affect the restoration of *Podocarpus cunninghamii* within this environment, with special attention paid to the role of arbuscular mycorrhizal fungi (AMF).

Field investigations of *Podocarpus cunninghamii* communities showed that they contain a high degree of floristic and structural variation determined by soil and climatic variables. Analysis of age and size class distributions suggest that *Podocarpus cunninghamii* has more than one regeneration strategy, and can regenerate within intact forest following the opening of small canopy gaps or can undergo large-scale recruitment following catastrophic disturbance. Field and glasshouse experiments investigating growth and nutrient responses of *Podocarpus cunninghamii* to different AMF inoculants found that *Podocarpus cunninghamii* responses are dependent on both AMF type and grass competition. Finally, investigation of *Podocarpus cunninghamii* carbon stocks showed that they are less than that of other New Zealand forest types, but are greater than that of grazed pastures.

Successful restoration of high country *Podocarpus cunninghamii* communities will require the incorporation of associated species based on local environmental conditions, and will also need to allow for disturbance processes. AMF may have an important role to play in restoration by reducing seedling production times and by increasing the competitiveness of *Podocarpus cunninghamii* when in competition with exotic grasses.

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1 Introduction

This thesis explores the ecology of *Podocarpus cunninghamii* in the eastern South Island high country and considers some of the factors that potentially affect its successful restoration. In this chapter I introduce both the eastern South Island high country and *Podocarpus cunninghamii*, and provide some background to the different elements of the thesis, as well as the overall thesis structure.

1.1 The eastern South Island high country

The South Island high country is a diverse and evocative landscape encompassing rugged mountains, intermontane basins, dense forest and golden tussock grassland. Much of the natural diversity found in the high country is largely driven by the domination of the climate by prevailing westerly winds rich in moisture. The Southern Alps, which form a near unbroken chain of mountains from the north to the south of the South Island, create a distinct physical barrier, causing the vast majority of the moisture carried by the prevailing wind to be deposited on the west coast, where annual rainfall values can exceed 10,000 mm (Wardle, 1991). Once east of the Main Divide there is a dramatic reduction in rainfall that follows a declining eastwards gradient; along this gradient rainfall can drop to as low as 350 mm per annum (MAF, 2001, Wardle, 1991), forming the "eastern New Zealand dryland zone", or more simply, the "drylands" (Rogers *et al.*, 2005). Continuing eastwards to the coast, the landscape becomes increasingly affected by easterly winds carrying moisture from the Pacific and Southern Oceans. As such, the east coast receives greater annual rainfall than much of the high country east of the Main Divide.

An approximate outline of the extent of the South Island high country is provided by Swaffield and Hughey (2001) (Figure 1.1). It can generally be broken down into three distinct geographical areas: the Western Barrier, the Eastern Range and Basin (ERB), and the Otago Block and Basin (OBB). The eastern South Island high country, to which this research is confined, is largely delineated by the boundaries of the ERB and OBB. These Blocks differ markedly from the Western Barrier due to the

dramatic rainfall gradient described earlier. The eastern boundary of the Western Barrier does not precisely follow the ranges of the Main Divide; as such, many

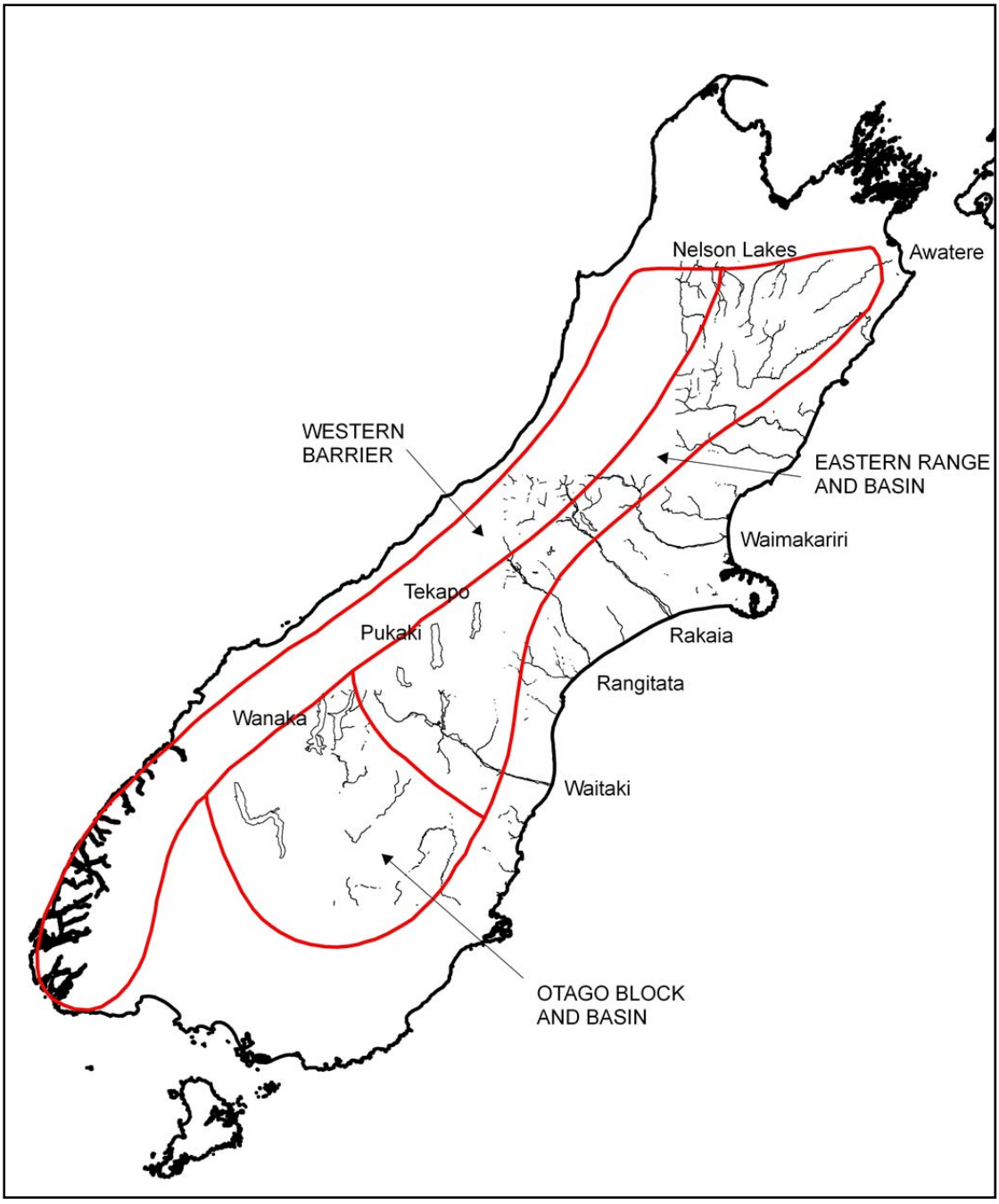

Figure 1.1 – The high country of New Zealand's South Island. The eastern high country is largely defined by the Eastern Range and Basin and the Otago Block and Basin. The eastern mountains of the Main Divide fall within the Western Barrier. Modified from Swaffield and Hughey (2001).

mountains that are actually part of ranges east of the Main Divide fall within the area defined as the Western Barrier. In addition, portions of the Seaward Kaikoura Range exist outside of the boundary of the ERB. Despite these limitations, the area bounded by the ERB and OBB largely defines the geographical scope of this research project; that is, the eastern South Island high country, which for the purposes of convenience, shall henceforth be simply referred to as 'the high country'.

Prior to human settlement, forest is believed to have covered 85-90% of the New Zealand land surface below the alpine tree line (McGlone, 1989). A combination of factors, most notably early Polynesian fires followed by further fire, forest clearing and the introduction of grazing animals associated with European agricultural expansion, led to a dramatic decline in this cover; New Zealand's indigenous forests now accounts for only 24% of the land cover (Ewers *et al.*, 2006). The dry eastern ranges of the South Island have been particularly vulnerable to this forest loss.

Since European discovery in the nineteenth century, the high country has had a long history of grazing management, with frequent burning to clear scrub and maintain pasture (Buchanan, 1868, Munro, 1868). The current vegetation therefore reflects those species that survived initial Polynesian deforestation followed by the more recent impacts of expansionist European pastoralism; the landscape is therefore predominantly grassland. Those woody species remaining are likely to represent the most fire tolerant and unpalatable members of the flora (Standish *et al.*, 2009). In addition, it is immediately discernable from cursory observation of the high country that remnant patches of woody vegetation have a non-random distribution – they find refuge within gullies, or the confines of steep boulder fields, or cling to rocky outcrops and bluffs; all of which are fire-free refuges. This combination of anthropogenic and refuge-based species selection makes it highly unlikely that these remnant patches of vegetation are representative of the flora that covered the landscape prior to human arrival. The small size of the remnants also makes them highly susceptible to edge effects, further diminishing their likelihood of representing the original flora (Walker *et al.*, 2004a).

Although the remnant woody communities present in the high country are unlikely to accurately represent the pre-human regional flora, they are still of great importance.

They are the last vestiges the pre-human woody flora, modified or not, thus providing invaluable insight into the vegetation history of the high country (Walker *et al.*, 2003). Furthermore, they are of great conservation value, providing the only indigenous habitat for woodland species within a highly deforested landscape – Marlborough, Canterbury and Otago have lost 74%, 91% and 90% of their pre-human forest covers respectively (Ewers *et al.*, 2006). While this forest loss has not been restricted to the high country, climatic moisture stress has been found to be highly correlated with forest loss (Ewers *et al.*, 2006). This moisture stress made the eastern dryland vegetation more vulnerable to fire than many eastern coastal areas that experience less moisture stress, and thus exacerbated the extent of loss in the eastern drylands (McGlone, 2001). Further emphasising the historical and conservation importance of these remnant communities, many of them also occur within "land environments" classed as either "At Risk" or "Critically Underprotected" (Walker *et al.*, 2008). "At Risk" environments are defined as having 20-30% of the estimated pre-human indigenous cover remaining; "Critically Underprotected" environments have >30% pre-human indigenous flora remaining but <10% is legally protected. In fact, only 1.9% of the entire dryland zone of New Zealand has legal protection (Walker *et al.*, 2009), and added to their vulnerability is our lack of knowledge of their ecology (Walker *et al.*, 2003).

In the last 10-20 years, increasing areas of the high country have changed tenure, and have been taken out of active pastoral production and placed under the management of the Department of Conservation (Mark *et al.*, 2009). This has released large tracts of former agricultural lands from grazing by domestic livestock (Rogers *et al.*, 2005). The removal of livestock grazing is widely considered conducive to the return of woody ecosystems to these landscapes (Molloy, 1989, Swaffield and Hughey, 2001). However, given the lack of knowledge pertaining to the ecology of indigenous high country woody ecosystems, it is recognised that there are many uncertainties associated with making decisions on how best to both manage and restore them (Rogers *et al.*, 2005).

1.2 *Podocarpus cunninghamii*

Podocarpus cunninghamii, known commonly as Hall's, mountain, paper-barked or thin-barked totara, is arguably New Zealand's most tolerant conifer, occurring in both lowland and upland environments, in moisture laden rainforest and semi-arid drylands. A member of the Podocarpaceae, it is a New Zealand endemic species able to attain heights of 15-20 m and trunk diameters of up to 2 m. Its needle-like foliage is pointed and rigid, often with a distinctive olive green colouration. The bark of mature trees forms a thick layer around the trunk and comes away in long paper-like strips (Dawson and Lucas, 2000, NZPCN, 2010). It is the subject species throughout this thesis.

It is worth clarifying the nomenclature used for this species in this thesis. Two names have been proposed: *Podocarpus cunninghamii* Colenso and *Podocarpus hallii* Kirk. The name *Podocarpus cunninghamii* was proposed five years before *Podocarpus hallii* (1884 versus 1889), and Kirk is said to have been aware of Colenso's name when he decided to name the species after Hall (Connor and Edgar, 1987). Kirk is regarded as having provided the first definitive description of the species, and it has been suggested that Colenso had no intention of ever publishing the name *Podocarpus cunninghamii*; based on this argument, it has been suggested that *Podocarpus hallii* is the correct name (Connor and Edgar, 1987). However, more recently it has been argued that *Podocarpus cunninghamii* is the correct name because 1) it does pre-date the name *Podocarpus hallii*; and 2) Kirk himself agreed that if *Podocarpus cunninghamii* and *Podocarpus hallii* were found to be the same species, then the name *Podocarpus cunninghamii* "must take precedence" (de Lange and Rolfe, 2010). In light of this, and despite the fact that the species is commonly referred to as *Podocarpus hallii*, both scientifically and more widely (e.g. in more popular books), I will use the name put forward by Colenso in this thesis, that is, *Podocarpus cunninghamii*.

Prior to human arrival, in the period since the mid-Holocene (c. 7,000 years Before Present (BP)) up to human arrival in New Zealand (c. 800 years BP (Wolfe and Klironomos, 2005, Callaway *et al.*, 2008)), forest covered extensive areas of the high country. There is consensus that historically a range of vegetation types existed,

including woody and non-woody types. Charcoal, fossil and palynological records all indicate that below tree line, on mid- to lower altitude mountain slopes, two forest types were the predominant forms of vegetation. Forests dominated by *Nothofagus* species were common, particularly along the high precipitation axial ranges north of the Rakaia headwaters up to the Kaikoura Ranges, and southwards from the head of Lake Pukaki (Leathwick, 2001). *Nothofagus* was absent from the area in between (covering the Rakaia and Rangitata catchments), known as the Southern Alps "beech gap" (Pivato *et al.*, 2009), and further to the southeast, in the dryland areas of the Mackenzie Country and Central Otago (Figure 1.2). These areas remained covered by forests in which *Podocarpus cunninghamii* was a major component (McGlone, 1989, McGlone, 2004, Molloy *et al.*, 1963). *Podocarpus cunninghamii* was the dominant podocarp in these communities and formed distinctive tall forests in association with species such as *Griselinia littoralis* and *Phyllocladus alpinus*. Both forest types were largely absent from valley bottoms due to the effects of diurnal temperature inversions and water stress; these bottomlands supported a small-leaved shrub and grassland community (McGlone, 2004). Extensive tracts of *Nothofagus* forest still exist, and much of these forests occur within New Zealand's public conservation estate, such as in Arthur's Pass National Park, Ahuriri Conservation Park and Mt Aspiring National Park. In contrast, *Podocarpus cunninghamii* has a very limited and scattered present day distribution throughout the high country (Norton, 2006), and many populations occur outside of the public conservation estate.

The woody communities of the high country are amongst the least understood and least studied ecological systems within New Zealand (Rogers *et al.*, 2005, Walker *et al.*, 2003). As a result, very little is known of the ecology of *Podocarpus cunninghamii* within the high country environment; for example, little is known of the vegetation communities *Podocarpus cunninghamii* forms and how composition is affected by environmental gradients. The study by Wells (1972) remains the best source of information on *Podocarpus cunninghamii* ecology in the high country, but it is limited to Central Otago and provides only scanty information on *Podocarpus cunninghamii* community ecology and population dynamics. In the absence of this knowledge it is not possible to develop an ecological basis for the restoration of *Podocarpus cunninghamii* populations within the high country.

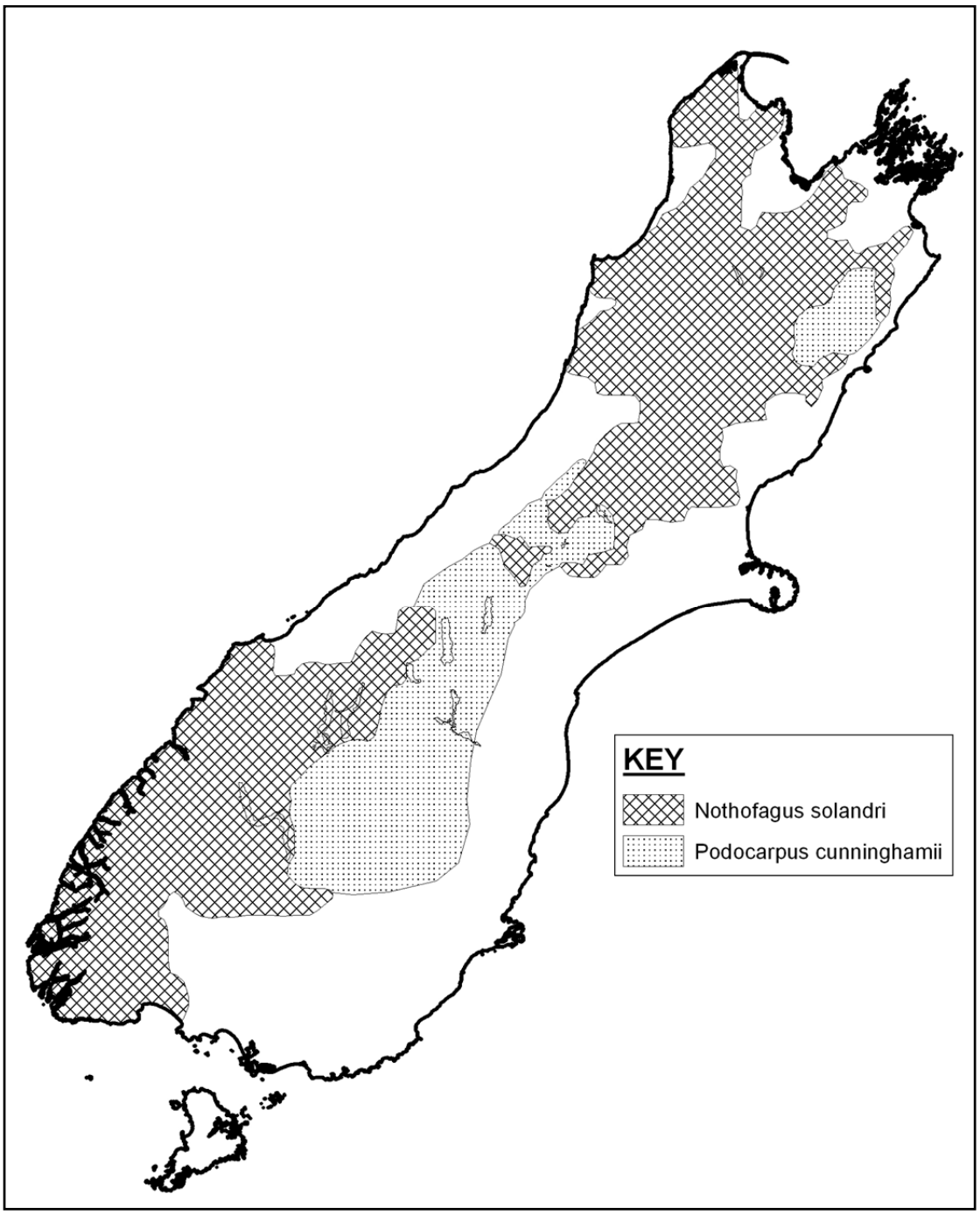

Figure 1.2 – The current distribution of *Nothofagus solandri* (MAF, 2009), and the areas east of the Main Divide where, prior to human arrival, *Podocarpus cunninghamii* forest is thought to have dominated and remnants still exist.

1.3 Why restore *Podocarpus cunninghamii*?

The question of 'why restore?' encompasses two related but different questions. The first is why should we attempt to restore *Podocarpus cunninghamii* communities? The second is, if we accept that we should restore these communities, why do we need to actively conduct ecological restoration rather than simply set aside the grasslands and allow them to naturally revert back to their historically wooded state?

1.3.1 Why we should restore *Podocarpus cunninghamii* forest

The ecological restoration of *Podocarpus cunninghamii* communities to parts of the high country offers numerous benefits to society. These benefits, which are common to all abandoned agricultural fields on which native woody vegetation redevelops, centre on the potential for improved conservation of indigenous biodiversity, control of soil erosion, and increased carbon sequestration (Standish *et al.*, 2009).

1.3.1.1 Conservation of indigenous biodiversity

The current distribution of *Podocarpus cunninghamii* remnants is both very limited and highly fragmented (Walker *et al.*, 2003). In light of the evidence for an extensive pre-human distribution of *Podocarpus cunninghamii* communities across the high country compared with the current distribution, it is clear that, in terms of habitat loss, *Podocarpus cunninghamii* communities are amongst the most impacted forest types in New Zealand. As such, the restoration of *Podocarpus cunninghamii* woody communities to the high country would be in of itself an important step for the conservation of indigenous biodiversity. However, because *Podocarpus cunninghamii* remnants are also highly fragmented, restoration can play another important role.

Fragmented populations are often small, and being subject to the species-area relationship they tend to have low species diversity (Meurk and Hall, 2006, Tate *et al.*, 1997). In addition, fragments typically exist within a highly modified landscape, e.g. a forest patch within an agricultural landscape. This subjects the fragment to edge effects, such as changes in wind exposure and water availability, as well as increased susceptibility to invasion by species found in the surrounding landscape matrix (Saunders *et al.*, 1991, Carswell *et al.*, 2008, Tate *et al.*, 1997). This includes native

species not typical of the fragment vegetation type but that occur adjacently, e.g. grassland species invading a forest fragment edge, as well as exotic species. Edge effects can alter the microclimate of the fragment and make it less suitable for its original forest inhabitants (Saunders *et al.*, 1991, Murcia, 1995). Depending on the shape and total area of the fragment, edge effects may pervade throughout the entirety of the remnant and remove all 'core' habitat, leading to changes in species composition and abundance (Kirschbaum *et al.*, 2009, Newsome, 1987). The isolation in which fragments often occur can also cause genetic bottlenecking and population decline through density dependent phenomena such as the Allee effect (Stephens and Sutherland, 1999, Gibbs *et al.*, 2007). Furthermore, models of climate change predict that species will need to migrate at historically unprecedented rates in order to keep pace with climate induced range shifts; this will likely have dire consequences for populations confined to isolated remnants within a highly modified landscape that inhibits both gene flow and migration (Davis and Shaw, 2001, Travis, 2003). In light of this, restoration of *Podocarpus cunninghamii* woody communities can increase both the connectivity of remnants and the size of existing remnants. This will enhance biodiversity conservation values within the high country and give greater resilience to populations of indigenous species in the face of any future perturbations, climatic or otherwise.

1.3.1.2 Soil erosion control

The conversion of high country forests to grassland has increased its susceptibility to soil erosion. Contrary to the findings of early studies on high country soil erosion, which were carried out in the early 1900s, the current view is that the majority of human-induced, or "anthropic" soil erosion took place as a result of early Polynesian deforestation rather than European pastoralism (McSaveney and Whitehouse, 1989, Whitehouse, 1984). However, it is considered that tussock grasslands exposed to heavy sheep grazing, frequent burning, rabbit infestation or *Hieracium* invasion are environments with high susceptibility to surface soil erosion (Hicks, 1995). Of most concern in the high country is erosion caused by wind blow (Plate 1.1) and sheet wash; 77%, 86% and 88% of soil erosion in Nelson-Marlborough, Canterbury and Otago, respectively, is driven by these processes, and the percent of farmland within these regions on erodible land, in 1995, was 31%, 69% and 58% respectively (Hicks, 1995). A proportion of this land is no longer affected by sheep grazing, due to

changes in land tenure, but rabbit infestation and ongoing herbivory by feral ungulates, as well as *Hieracium* invasion, remain serious problems that maintain the vulnerability of these areas to wind erosion.

Plate 1.1 – Wind-borne soil erosion near Omarama, south Canterbury.

The restoration of woody communities to deforested hill country has been found to have beneficial effects in terms of reducing soil erosion. Research in the Loess Plateau, China, an area that suffers from high levels of soil erosion and which has similar annual precipitation to the high country (560-700 mm), has shown that reforestation of hill country can result in significant reductions in soil erosion rates (Li *et al.*, 2010, Zheng, 2006). This suggests that the restoration of *Podocarpus cunninghamii* communities in the high country could also reduce rates of soil erosion, especially if it was targeted to particularly erosion prone areas like steep gullies or severely degraded hill faces (O'Loughlin, 2005).

1.3.1.3 Carbon sequestration

As part of the global effort to confront climate change, numerous countries became signatories to the Kyoto Protocol (UNFCC, 1998). Under the conditions stipulated within the Protocol, participating countries are required to reduce their carbon emissions, and are able in part to do so by offsetting their emissions through afforestation. Many high country grasslands can be classed as marginal, having low productivity values and soils susceptible to erosion (Davis *et al.*, 2009, Kirschbaum *et al.*, 2009, Millenium Ecosystem Assessment, 2005). Afforestation of these grasslands, which cover hundreds of thousands of hectares, would contribute significantly to New Zealand achieving its emissions reduction commitments (Carswell *et al.*, 2008, Kirschbaum *et al.*, 2009). Although New Zealand's indigenous forest species have slower growth rates than some exotic conifer species, and therefore lower initial rates of carbon sequestration, if left unharvested they do have the potential to develop greater long term carbon stocks than conventionally managed exotic production forests (Carswell *et al.*, 2008, Chazdon, 2008). In light of this, and given the marginal nature of the soils, indigenous afforestation may be a preferred option in the high country. If so, the prospect of increased carbon sequestration may act as a driver for *Podocarpus cunninghamii* restoration.

1.3.2 Why we need active restoration of *Podocarpus cunninghamii* forest

As already stated, large tracts of the high country have recently been placed within New Zealand's public conservation estate. This change of land tenure marks the beginning of the conservation process by permitting the removal of some of the factors attributable to maintaining the predominantly grassy nature of the high country below tree line. However, change of land tenure *per se* is not the same as changing management and achieving conservation goals (Norton, 2006). While transfer of land tenure to the public conservation estate allows the removal of degrading factors such as domestic livestock grazing, fire management and regular fertiliser application, it does not address fundamental changes that these degrading factors have over time effected (Cramer *et al.*, 2008, Standish *et al.*, 2009), and therefore, by itself does not guarantee the return of the desired indigenous flora (Walker, 2000). For example, the removal of grazing by domestic livestock or the cessation of fertiliser application will

not result in the automatic reversion of the high country back to *Podocarpus cunninghamii* dominated communities (Norton, 2006).

The reason such reversion will not occur is due to a combination of threshold dynamics and the impact of invasive species (Hobbs and Norton, 2004, Standish *et al.*, 2009, Norton, 2009). For example, the high country will not automatically revert to *Podocarpus cunninghamii* forest because of the sheer lack of seed sources (Norton, 2006, Standish *et al.*, 2009); a biotic threshold. In addition, the destruction and loss of *Podocarpus cunninghamii* forest has reduced the amount of habitat available for *Podocarpus cunninghamii* seed dispersers, such as bellbird (*Anthornis melanura*); this acts as a positive feedback further hindering the reversion of the landscape back to its original vegetation (Suding and Hobbs, 2009b). Furthermore, the total number of indigenous species that are available to recolonise the high country does not include those that were filtered from the original, pre-human species pool by the effects of fire, grazing and competition with invasive species (Hobbs and Norton, 2004, Standish *et al.*, 2009). It is also worth remembering that the removal of domestic livestock grazing does not equate to the cessation of herbivory, due to the large numbers of lagomorphs and feral ungulates present in the high country that exist independently of pastoral farming (Standish *et al.*, 2009). It is also possible that these independent herbivores could even increase in abundance in the absence of domestic livestock in response to the removal of resource competition.

Movement of high country systems across abiotic thresholds is also likely to have occurred, especially in the dryland zone where agriculture was environmentally limited due to low annual rainfall and low temperatures (Cramer *et al.*, 2008). Long lasting legacy effects of agriculture, such as changes in soil hydrology and fertility, can require substantial human efforts to overcome before indigenous species can re-establish (Cramer *et al.*, 2008, Standish *et al.*, 2009). For example, efforts that improve native woody species establishment in exposed high country grasslands have included the creation of sheltered microsites (Paul and Ledgard, 2008). Therefore, unless both these biotic and abiotic threshold changes are addressed, the removal of degrading factors alone will not result in natural recolonisation of *Podocarpus cunninghamii* forest. A further reason why automatic reversion to the pre-human state will not occur is the presence and effect of exotic invasive species. Introduced flora

such as *Agrostis capillaris*, *Hieracium* spp. and exotic conifers are now nearly ubiquitous throughout the high country and can compete effectively for soil nutrients and water (Espie, 2001, Ledgard, 2001, Walker *et al.*, 2009). It will be impossible to eradicate these species with current technology and it is likely that any *Podocarpus cunninghamii* forest that develops, whether naturally or through active restoration, will contain elements of these exotic species (Norton, 2009, Standish *et al.*, 2009). This will result in the establishment of stable novel ecosystems, with varying mixes of indigenous and exotic species (Suding and Hobbs, 2009a, Walker *et al.*, 2009).

In light of the need for active efforts to overcome restoration thresholds, in addition to the removal of degrading factors, it is vital that both the basic ecology of *Podocarpus cunninghamii* in the high country as well as the thresholds that hinder attempts at restoration are better understood (Suding and Hobbs, 2009b). It will be impossible to restore the landscape to the pre-human flora for several reasons, including the fact that it is unknown what the original flora actually consisted of and the pervasive impact of invasive species (Norton, 2009). However, understanding what species currently form associations with *Podocarpus cunninghamii* and how their abundance changes along environmental gradients forms a baseline for current community status, i.e. provides a measure of how 'intact' the different communities are, and what the prevalence is of exotic species. From this, sites with similar environmental conditions and species composition can be grouped, allowing community composition and structure to be predicted for particular site types. Understanding *Podocarpus cunninghamii* population dynamics will also be important for determining tree establishment and regeneration pathways (Norton, 1983, Veblen and Stewart, 1982), and hence to understand likely long term trajectories of both restoration plantings and remnant areas.

The requirements for successful restoration also demand investigation. A previous trial investigating *Podocarpus cunninghamii* early establishment success in the high country, in the Mackenzie Basin, have recorded survival rates as low as 29% after four years (Ledgard, 2004) and 100% mortality after six years (N. Ledgard, pers. comm.). Given the cost of individual *Podocarpus cunninghamii* seedlings (approximately $5), this level of survival has to be improved. Two avenues, founded upon the concept of threshold dynamics, are of particular relevance: mycorrhizal

fungi and nurse plants. For completeness, an overview of both follows, but the thesis goes on to focus specifically on arbuscular mycorrhizal fungi, with only occasional reference to nurse plants.

1.3.2.1 Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) (phylum Glomeromycota (Schüβler *et al.*, 2001)) are a distinct group of soil fungi that colonise the roots of approximately two-thirds of all terrestrial vascular plants (Smith and Read, 2008). They play a major role in plant acquisition of nutrients, particularly phosphorous (Baylis, 1972); they improve both plant-soil water relations (Augé, 2001) and soil aggregation (Rillig and Mummey, 2006); and can provide plants with protection from soil pathogens (Whipps, 2004). As a result they can alter the competitive relationship between plants (Scheublin *et al.*, 2007) and have been found to be a major determinant of plant community diversity (van der Heijden *et al.*, 1998b). Their potential importance within ecological restoration is therefore gaining increasing attention (Renker *et al.*, 2004). The vast majority of New Zealand's indigenous flora forms symbiotic associations with AMF, and *Podocarpus cunninghamii* is no exception (Baylis, 1969, Johnson, 1977).

Arbuscular mycorrhizal fungi are known to have low host specificity in terms of being able to colonise a wide variety of host species, but high specificity in terms of the effect they have on the host (van der Heijden *et al.*, 1998b). Importantly, the AMF-plant symbiosis lies on a continuum from mutualism to parasitism (Johnson *et al.*, 1997). It has been demonstrated that AMF diversity within agricultural systems is, in general, low compared to woodland systems, and that AMF genera most abundant in woodland systems are all but absent in agricultural systems (Helgason *et al.*, 1998, Daniell *et al.*, 2001). In light of this, the ex-agricultural high country grasslands now set aside for conservation may harbour AMF communities very different to those that occur within indigenous forest. While the AMF community present within ex-agricultural fields will almost certainly be able to colonise *Podocarpus cunninghamii*, there is no guarantee it will provide any host benefit, or even that it will not be parasitic. Lack of suitable mycorrhizal inoculum has been highlighted as a potential constraint to the successful establishment of native forest in abandoned agricultural

fields (Holl *et al.*, 2000, Standish *et al.*, 2009), and may therefore represent a biotic restoration threshold.

Arbuscular mycorrhizal fungi are also increasingly available for purchase within commercial products and are used within plant nurseries. These are typically based on easy-to-culture AMF species from a particular location within a single country. When sold on a non-local scale, e.g. internationally, these products form novel mycorrhizal associations with the indigenous flora being propagated (Mummey *et al.*, 2009, Schwartz *et al.*, 2006). The use of exotic AMF does not always result in increased growth of native plants (Caravaca *et al.*, 2003, Requena *et al.*, 2001, Richter and Stutz, 2002), and as such there is increasing interest in the application of indigenous AMF for ecological restoration, both within New Zealand (Williams, 2009) and internationally (Corkidi *et al.*, 2008). Furthermore, exotic AMF can have negative consequences for realising the diversity of AMF found within natural plant communities (Mummey *et al.*, 2009), and increases the possibility of introducing invasive species (Schwartz *et al.*, 2006). These are important considerations for ecological restoration.

1.3.2.2 Nurse plants

The facilitative effects of cover or nurse plants for tree establishment within dry grassland environments has been demonstrated numerous times (e.g. Gómez-Aparicio *et al.*, 2004, Li and Wilson, 1998, Rousset and Lepart, 1999). The reason for this facilitative effect tends to centre on the nurse plant providing favourable microsite conditions for improved seed germination and/or improved seedling survival and establishment. These improved conditions can take the form of increased soil moisture and nutrient availability, frost protection, shade from intense solar irradiation, and protection from the trampling effects of large grazing animals (Padilla and Pugnaire, 2006).

Within the high country dryland zone, shading or shelter has been found to have a positive effect on soil moisture values and in improving the establishment of native woody species (Paul and Ledgard, 2008, Payne and Norton, 2010). However, any facilitative effect may be dependent on the form of cover, as *Podocarpus cunninghamii* seedling mortality within a high country restoration trial were greatest

within a stand of exotic conifers used as nurse plants compared with those grown in open grassland (Ledgard, 2004). Nurse plants may also enhance restoration by providing suitable habitat for seed dispersing birds to forage and perch within (Reay and Norton, 1999), provided that seed sources exist nearby.

1.4 Aims and objectives

The aims of this thesis are to gain a better understanding of the ecology of *Podocarpus cunninghamii* dominated communities in the eastern South Island high country, and to investigate factors potentially affecting the species' restoration within this environment, with special attention to the role of AMF. Together, these investigations should allow the development of a stronger ecological basis for *Podocarpus cunninghamii* restoration within the high country. Specifically, the objectives are as follows:

- Define the vegetation communities that *Podocarpus cunninghamii* forms throughout the high country, identifying key associations and how community composition and structure varies along environmental gradients.
- Investigate *Podocarpus cunninghamii* population dynamics in the high country, by examining the age and size structure of several stands along the rainfall gradient.
- Investigate the mycorrhizal associations of *Podocarpus cunninghamii* and adjacent grasslands using molecular methods.
- Determine *Podocarpus cunninghamii* early growth responses to colonisation by ex-agricultural versus forest AMF communities, and how this is affected by competition with exotic grass.
- Determine *Podocarpus cunninghamii* early growth responses to colonisation by an indigenous versus exotic, commercially available AMF, and how this is affected by competition with exotic grass.
- Identify current native plant nursery practice with regard to the use of mycorrhizal fungi.
- Present a first estimate of the carbon stocks associated with existing and potential future high country stands of *Podocarpus cunninghamii*.
- Provide practical recommendations for *Podocarpus cunninghamii* restoration

1.4.1 Thesis outline

The next chapter introduces *Podocarpus cunninghamii* more thoroughly, outlining the vegetation history of the high country in further detail and describing where *Podocarpus cunninghamii* forest remnants still exist. Chapter 3 investigates the vegetation communities associated with *Podocarpus cunninghamii* together with *Podocarpus cunninghamii* population dynamics. These chapters provide the baseline data of the ecology of *Podocarpus cunninghamii* in the high country. Chapters 4-9 focus on restoration thresholds and mycorrhizal associations. Chapter 4 provides a literature review of AMF and their potential role in forest restoration on ex-agricultural fields; Chapter 5 is a molecular investigation of the AMF communities present within *Podocarpus cunninghamii* forest and adjacent grasslands; Chapters 6-8 investigate how *Podocarpus cunninghamii* responds to different AMF treatments within both glasshouse and field environments; Chapter 9 elucidates current native plant nursery practice with regard to the use of AMF. The basis for Chapter 10 is also found in restoration, but rather in restoration incentives rather than thresholds, and investigates existing and potential future carbon stocks within high country stands of *Podocarpus cunninghamii*. Chapter 11 attempts to synthesise the preceding chapters, draw conclusions from the research and highlight further avenues of potential investigation.

2 *Podocarpus cunninghamii* in the eastern South Island high country[1]

2.1 Introduction

Before human arrival in the South Island, the high country below tree line and above the valley bottoms was covered in two forest types: *Nothofagus* forest and podocarp-broadleaf forest (McGlone, 1989, McGlone, 2004). *Nothofagus* forest dominated the high precipitation mountain slopes along the majority of the axial ranges, from north of the Rakaia catchment up to the Kaikoura Ranges, and south from the head of Lake Pukaki all the way to Fiordland (Figure 1.2). The area between the Rakaia catchment and the head of Lake Pukaki is known as the "Southern Alps beech 'gap'" (Hall and McGlone, 2006). Here *Nothofagus* was largely absent, and podocarp-broadleaf forests dominated. Podocarp-broadleaf forest also dominated in the areas further to the southeast, in the dryland areas of the Mackenzie Country and Central Otago (McGlone, 1989, McGlone, 2004). Similarly, podocarp-broadleaf forest dominated much of the central portion of Kaikoura Ranges, where *Nothofagus* was absent (McGlone and Basher, 1995). *Podocarpus cunninghamii* was a major component of these podocarp-broadleaf forests, and was the dominant podocarp species (McGlone, 1989, McGlone, 2004, Molloy *et al.*, 1963). It is these *Podocarpus cunninghamii* dominated communities, that existed in the areas where *Nothofagus* has historically been absent, that are the focus of this chapter.

As a consequence of early Polynesian and later European fires, together with the introduction of grazing animals associated with European agricultural expansion, the cover of these forests has been greatly diminished, with the greatest loss occurring in the dry eastern ranges (Ewers *et al.*, 2006). Because the greatest loss occurred in the drylands, the cover of *Podocarpus cunninghamii* forest has been massively reduced, while extensive stands of *Nothofagus* remain (see Chapter 1). The current distribution of *Podocarpus cunninghamii* remnants is both very limited and highly fragmented (Walker *et al.*, 2003). The current distribution is also uneven, with remnant stands

[1] This chapter has been submitted to the *Journal of the Canterbury Botanical Society*: Williams, A. *Podocarpus cunninghamii* in the eastern South Island high country.

being more intact or greater in extent within the wetter parts of high country, and more degraded or covering a smaller area in the drier parts. However, despite the extent of loss of this historically common vegetation type, together with its highly fragmented current distribution, information on its ecology is scanty. Furthermore, information pertaining to the current distribution of *Podocarpus cunninghamii* communities is distributed across numerous publications, many of which belong to the grey literature. As such, information relating to the ecology and distribution of *Podocarpus cunninghamii* within the high country is limited and often difficult to find.

This chapter provides an overview of the vegetation history of the eastern South Island high country, as it relates to *Podocarpus cunninghamii* dominated vegetation communities. Following this, the chapter summarises the numerous records of *Podocarpus cunninghamii* in the high country, and identifies and describes the remnant patches of *Podocarpus cunninghamii* communities that remain.

2.2 Vegetation history of the eastern South Island high country

2.2.1 Pre-human cover

The contemporary landscape of much of the *Nothofagus*-free high country, especially the drier parts, notably the Inland Kaikoura Range, South Canterbury and Central Otago, are characterised by tussock grasslands. Fragments of *Podocarpus cunninghamii* dominated communities occur very sporadically and in isolation. This situation is very different to that in pre-human times, as evidenced by plentiful "totara" log remains and palynological records that indicate a formerly woody landscape. Log remains have been well documented across inland Marlborough, Canterbury and Central Otago for over 100 years (Buchanan, 1868, Cockayne, 1928, Munro, 1868, Speight, 1910, Wells, 1972). In addition to the fallen logs, less conspicuous evidence of historical forest cover has also been discovered in the form of charcoal deposits. Carbon dating of these log and charcoal remains indicates that between c. 12,000 – 10,500 years BP (McGlone, 2004) and the arrival of humans (c. 800 years BP (Higham *et al.*, 1999, McGlone and Wilmshurst, 1999)), *Podocarpus cunninghamii* was a dominant tree throughout the high country, especially between

Lakes Wanaka and Wakatipu, throughout the Mackenzie Country, and stretching northwards into the headwaters of the Rangitata and Rakaia Rivers, where plentiful remains have been found (Molloy *et al.*, 1963). Charcoal deposits become rarer north of Porters Pass but reappear along the Awatere River, on the western faces of the Inland Kaikoura Range (McGlone and Basher, 1995, Molloy *et al.*, 1963).

However, the heartwood of species such as *Podocarpus cunninghamii* and the associated conifer *Phyllocladus alpinus* is very strong and durable, and as such charcoal deposits and fallen log remains offer only a very selective view of the high country's historical forests; they could be taken to imply a near monoculture forest of *Podocarpus cunninghamii* and *Phyllocladus alpinus* covered the mountainsides of the high country outside the range of *Nothofagus* (Wardle, 2001b). The wood of other species that may well have co-occurred with these conifers (e.g. *Cordyline, Hoheria* and *Sophora*) do not preserve well and are thus underrepresented in such reconstructions. Palynology, the study of spores and pollen, though also selective, offers a more complete view of historical vegetation patterns (Pocknall, 1982). In addition, the palynological records studied have allowed insight further back in time than charcoal and log remains, providing a more complete view of vegetation history since the end of the last glacial maximum.

Investigations of pollen deposits have found that immediately following the end of the last glaciation (c. 15-12,000 year BP), when temperatures were warming following the glacial maximum, the vegetation of the high country was largely gramineous, with Poaceae pollen dominant among the palynological records (Burrows and Russell, 1990, McGlone *et al.*, 2004, Moar, 1971, Moar, 1980; Figure 2.1). Over the next few thousand years (13-10,000 years BP) the climate continued to warm and these grasslands were invaded and largely replaced by *Coprosma* shrublands with *Phyllocladus* (Burrows and Russell, 1990, Burrows *et al.*, 1993, McGlone *et al.*, 1995, McGlone *et al.*, 2004, Moar, 1971; Figure 2.1). Eventually, between 10-8,000 years BP, these shrublands were themselves replaced by podocarp dominated forest below tree line from the Inland Kaikoura Range down to the Mackenzie Basin (Burrows *et al.*, 1993, Burrows and Russell, 1990, McGlone and Moar, 1998, McGlone *et al.*, 1993, McGlone *et al.*, 2004, McLea, 1996, Moar, 1971; Figure 2.1); this vegetation transition took place later in Central Otago, circa 7,500 years BP (McGlone *et al.*,

1997; Figure 2.2). At this time the tussock grasslands that now dominate much of the high country landscape were largely restricted to alpine areas; below this their populations would have been localised around pockets of particularly infertile soils

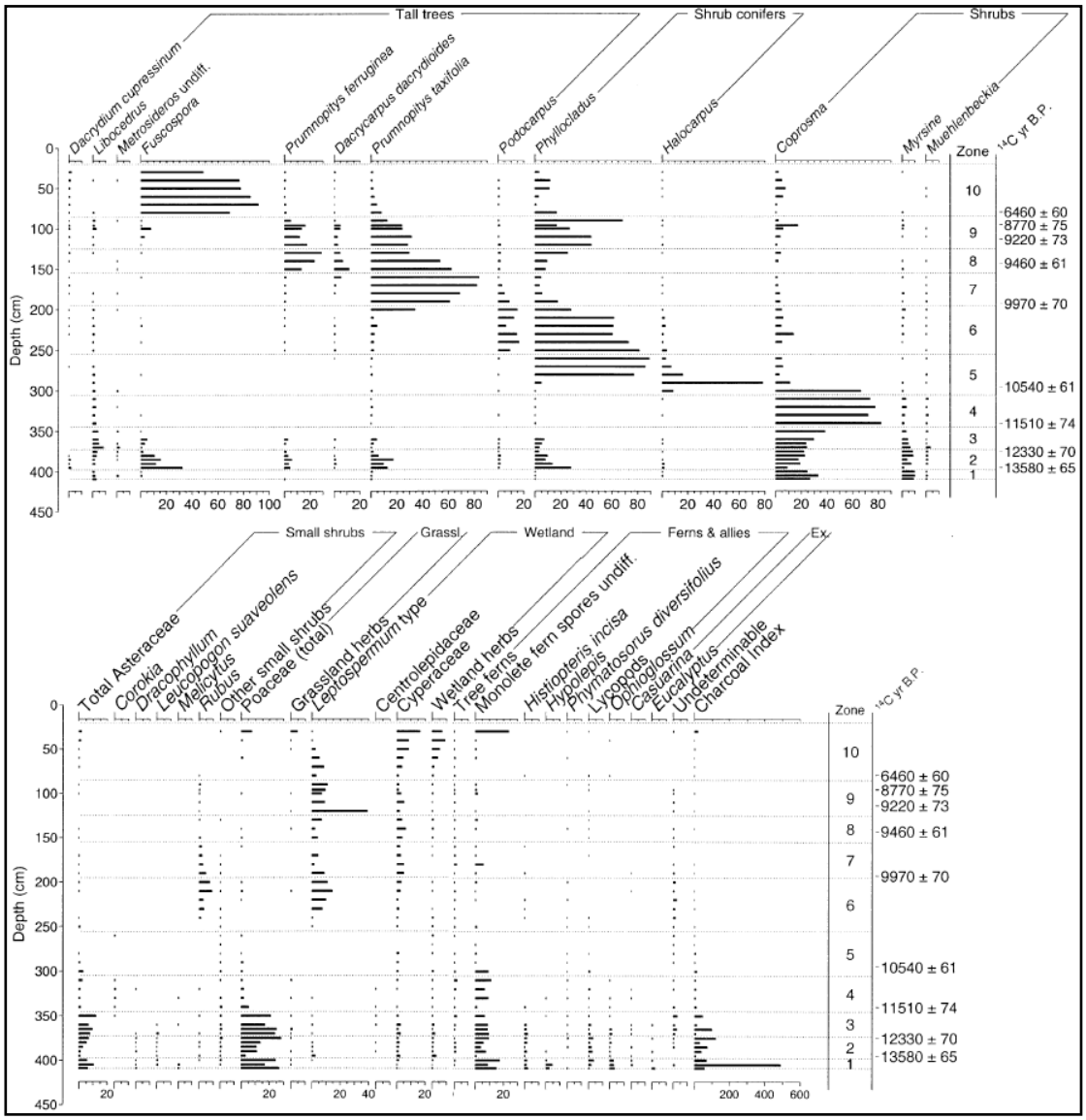

Figure 2.1 – Pollen diagram from Kettlehole Bog, Cass, Canterbury. The succession from Poaceae to *Nothofagus* (Fuscospora) via shrubland and podocarps is clear. Years before present are shown on the right (modified from McGlone *et al.*, 2004).

and bogs, valley bottoms, and recently disturbed sites (McGlone, 1989, McGlone, 2001). Small-leaved shrub communities too, for the most part, were confined to the valley bottoms and river terraces, and at the higher reaches of the sub-alpine zone (McGlone, 1989). Instead, in the wetter regions, such as in the headwaters of the

Rakaia and Rangitata Rivers, the heads of the lakes from Tekapo to Wakatipu, and the Seaward Kaikoura Range, *Podocarpus cunninghamii* formed a tall forest community with *Libocedrus bidwillii* (>2,500 mm annual rainfall), *Phyllocladus alpinus, Griselinia littoralis, Hoheria lyallii* and other angiosperm tree and shrub species (McKelvey, 1984, Veblen and Stewart, 1982). As the climate became increasingly arid, along the rainfall gradient, *Podocarpus cunninghamii* remained present, but in a low forest form, projecting above a canopy of increasingly scrub-form *Phyllocladus alpinus* and *Griselinia littoralis*. *Libocedrus bidwillii* became absent while *Sophora microphylla* and *Pittosporum tenuifolium* became more common. *Halocarpus bidwillii* would have formed a unique scrub community on the most extreme sites (McGlone, 1989, McGlone, 2004).

In the mid-Holocene, at approximately 7-6,000 years BP, the climate began to cool, with increasing incidence of more extreme weather events (droughts and snowfalls), strong winds and evidence for an increase in fire frequency. This change favoured *Nothofagus*, which had been present throughout the period of podocarp dominance but with a very limited distribution. All along the axial ranges of the South Island, excluding the central portion of the island, *Nothofagus* came to dominate and replaced the podocarp forests (McGlone, 2004, McGlone *et al.*, 1995, McGlone and Moar, 1998, McGlone *et al.*, 2004; Figure 2.1). This expansion again took place later in Central Otago, at around 7-3,000 years BP (McGlone *et al.*, 1997). Charcoal deposits also provide evidence of the invasion of wetter areas formerly dominated by *Podocarpus cunninghamii* by *Nothofagus* following burning events, such as the heads of Lakes Ohau and Hawea (Molloy *et al.*, 1963).

Nothofagus replaced podocarp forest in much of the high country; however *Podocarpus cunninghamii* communities remained the dominant vegetation type in the central portion of the South Island (e.g. the Rakaia catchment (Wardle, 1964)), along the drier mountains stretching east from the Main Divide (McGlone, 1989, McGlone, 2004; Figure 2.2) and in the central parts of the Inland Kaikoura Range (McGlone and Basher, 1995; Figure 1.2). The reason for the beech gap, where *Nothofagus* has not replaced *Podocarpus cunninghamii* forest in the central South Island, has not been fully resolved. Initially it was thought that during the last glacial maximum, *Nothofagus* found northern and southern refugia outside of this region (Wardle,

1963), and that as the climate gradually changed in its favour, *Nothofagus* has spread but only slowly, and has yet to realise the full extent of its potential geographic range (Wardle, 1964). Models of historical forest cover based on climatic tolerances of species are in agreement with this hypothesis, predicting *Nothofagus* forest across much of the beech gap (Hall and McGlone, 2006, Leathwick, 1998). Scarcity of suitable ectomycorrhizal symbionts has also been proposed as a potential reason for the slow spread of *Nothofagus* into the predominantly arbuscular mycorrhizal podocarp forests (Baylis, 1980). However, Hall and McGlone (2006) suggest that observations of scattered *Nothofagus* stands bordering the beech gap (Wardle, 1984) indicate that *Nothofagus* was not completely excluded during the last glaciation. Hall and McGlone (2006) also state that there is "little current physiological or distributional information for forest species relevant to this area, which is the driest, most drought-prone, and most seasonal in New Zealand". As such, they may not have been able to provide their model with the necessary information to accurately predict the species composition of historical forest cover in this area.

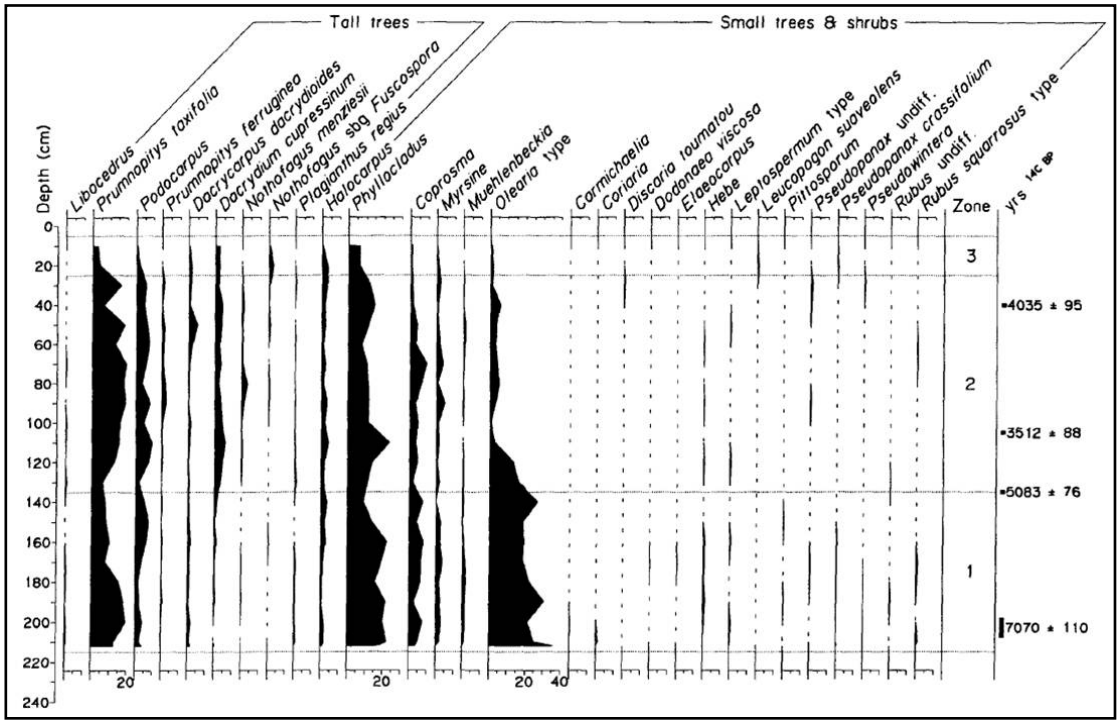

Figure 2.2 – Pollen diagram from Central Otago. The initial shrubland expansion was eventually succeeded by podocarp forest (from McGlone and Moar, 1998).

Despite the limitations of the models by Leathwick (1998) and Hall and McGlone (2006), other modelled predictions of historical forest cover have produced results similar to that suggested by log, charcoal and palynological data. In the model produced by Leathwick (2001), *Nothofagus* forest is predicted to have covered the high precipitation axial ranges except for the central part of the South Island, where conifer forests containing *Podocarpus cunninghamii* dominated. In the driest parts of the high country, in Central Otago, Mackenzie Country and the Inland Kaikoura Range, *Podocarpus cunninghamii* forest is predicted to have been the dominant cover, as indicated in Figure 2.3. Interestingly, *Podocarpus cunninghamii* and *Griselinia littoralis* were also found to have optima on sites with minimal soil water deficit and reached greatest abundance on steep to very steep, moderate to well-drained sites. This finding is somewhat at odds with their known localised abundance on very dry sites today.

The method employed by Leathwick (2001) is highly informative, especially when associated with extensive and relatively intact tracts of vegetation. In terms of canopy species composition, this approach shows close similarity to vegetation records published by McKelvey (1984) and Wardle (2001a) within high precipitation areas at the heads of the Wilberforce and Makarora rivers, respectively. However, there are limitations in the use of this method and it is inherently risky to use these models alone to extrapolate from relictual vegetation patches. This is because these relicts often exist within steep or sheltered sites that are not necessarily representative of the wider landscape, and, in addition, such remnant populations are typically small and isolated, thus they suffer from edge effects and can be highly modified (Walker *et al.*, 2004a).

In order to make more accurate predictions of historical woody species distributions prior to human arrival, within Central Otago, Walker *et al.* (2004a) utilised existing species distributions in conjunction with sub-fossil evidence (pollen spectra, log and charcoal remains) and the presence of plant life-forms within sites of environmental stress. Their analysis split Central Otago into 12 pre-settlement vegetation zones based upon environmental variables. According to the models, *Podocarpus cunninghamii* was expected to have dominated the more arid mid-elevation slopes between approximately 500-800 m above sea level (zones IV and V). *Nothofagus*

spp., *Sophora microphylla*, *Leptospermum scoparium*, *Kunzea ericoides*, *Griselinia littoralis* and *Phyllocladus alpinus* would also have been important components with *Podocarpus cunninghamii* depending on local site conditions. *Podocarpus cunninghamii* would have been replaced by *Nothofagus* on moister sites and by *Sophora microphylla*, *Leptospermum scoparium* and *Kunzea ericoides* on the lower slopes and valley bottoms. It is likely that some of these predictions could be extrapolated to the Mackenzie Basin, given its similarity in dryness to Central Otago, although *Nothofagus* was largely absent.

Overall, these models and records provide a picture of the changing flora of the high country prior to human arrival, from the end of the last glacial maximum to approximately 1,000 years BP. This picture shows that at the arrival of Polynesians, the majority of the high country below tree line and above the valley bottoms was forested. *Nothofagus* forest covered the axial ranges, excluding the central part of the South Island where *Podocarpus cunninghamii* forest flourished. Further east from the Main Divide in the drylands of Central Otago, the Mackenzie Country and the Inland Kaikoura Range, *Podocarpus cunninghamii* communities continued to be the predominant vegetation type.

2.2.2 Human impacts on the vegetation

The arrival of humans to New Zealand brought massive changes to the country's vegetation. It is now well established that early Polynesian fires wrought large-scale destruction of forest areas, particularly within dry, fire-prone sites (McGlone, 1989, McGlone, 2004). These changes were soon followed by the arrival of European pastoralists, who cleared many the woody communities that remained in order to establish sheep runs.

In pre-human times, fires in New Zealand were rare events. Lightning strikes, which are the only likely source of natural fires in the South Island, are rare in New Zealand compared with other countries (McGlone, 2001). Lightning strikes that do occur are most commonly restricted to the west coast, which may receive at most 20 electric storms each year (Ogden *et al.*, 1998). In addition, the return time of fire to the same

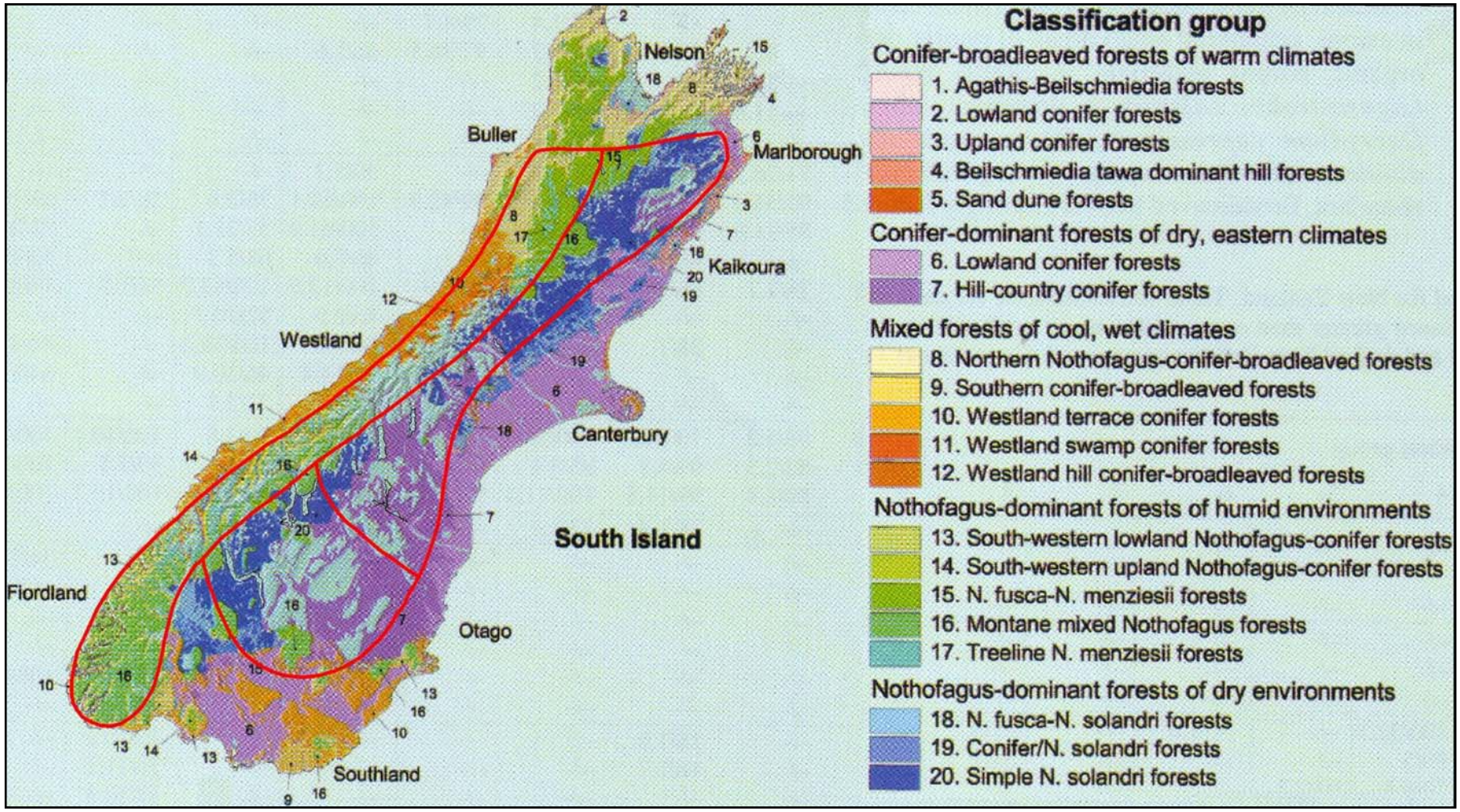

Figure 2.3 – Predicted potential pre-human forest cover of the South Island (modified from Leathwick, 2001). The outline of the high country (Swaffield and Hughey, 2001) is shown in red. The area defined as hill-country conifer forest (class seven, dark purple) is where *Podocarpus cunninghamii* forest is predicted to have occurred and remnants remain.

site has been measured at between 1,500 and 2,000 years, in pre-human times (Ogden *et al.*, 1998); meaning naturally burnt sites would have returned to forest before being re-burnt and would also have developed very large fuel loads. As a result of this fire rarity, the flora has not evolved adaptations to fire, and with few exceptions all of New Zealand's tree species are killed by fire (Ogden *et al.*, 1998, Wardle, 1991). Radiocarbon dating of available charcoal remains has found a massive increase in South Island fires beginning circa 800 years BP (McGlone and Wilmshurst, 1999, Ogden *et al.*, 1998, Rogers *et al.*, 2007). This analysis puts the sudden change of fire regime within the period of Polynesian settlement (McGlone and Wilmshurst, 1999, Ogden *et al.*, 1998). Furthermore, the increase in charcoal remains coincides with abrupt changes in palynological deposits. Sub-fossil evidence recovered from sites throughout the high country, from the Inland Kaikoura Range to Central Otago, shows a dramatic increase in Poaceae pollen and *Pteridium esculentum* spores between 700 and 500 years BP (c. 1300-1500 AD; Figure 2.4). These increases are concurrent with a marked decline in podocarp pollen (McGlone, 2001, Burrows *et al.*, 1993, McGlone *et al.*, 1997, McGlone and Basher, 1995, McGlone *et al.*, 1995, McGlone and Moar, 1998, Rogers *et al.*, 2007).

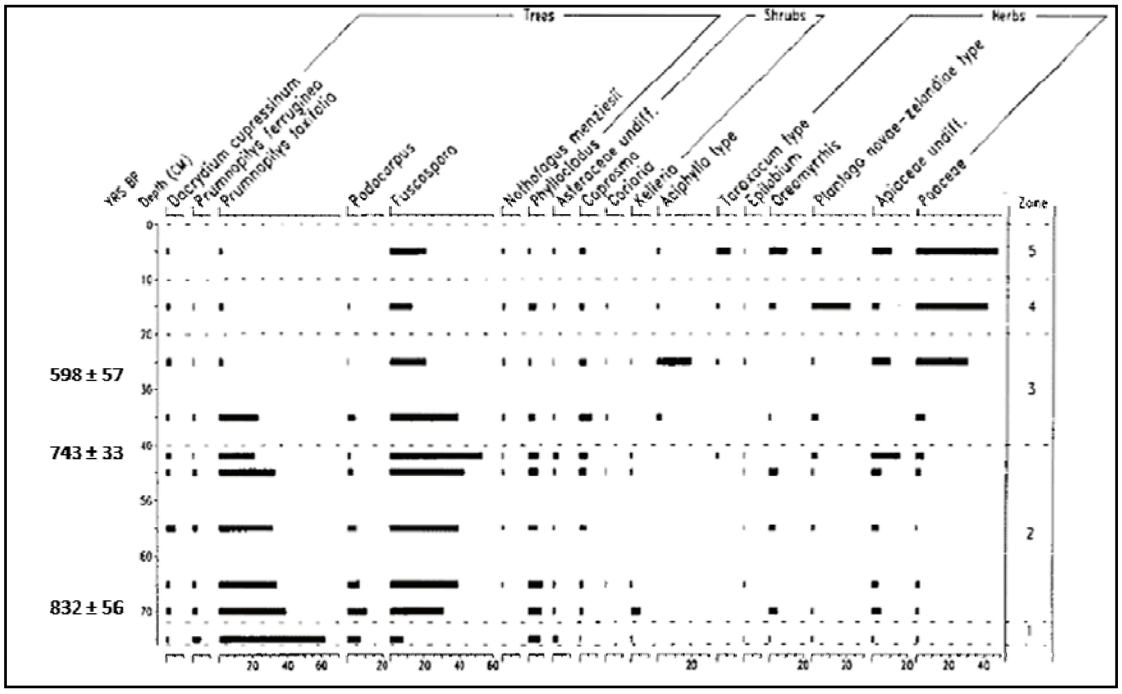

Figure 2.4 - Pollen diagram from Winterton Bog, Inland Kaikoura Range, Marlborough. Years before present are shown on the left (modified from McGlone and Basher, 1995).

It has been suggested that these conflagrations, the "Fires of Tamatea", were the result of deliberate and repeated land clearances by Maori, most likely to improve ease of travel and to favour the regeneration of starch-rich species, e.g. *Pteridium esculentum* (McGlone, 2004). A combination of the flammability of the vegetation and the accumulated fuel load, particularly within the dry eastern ranges, and the effect of the hot, dry föhn winds prevailing from the northwest, allowed the fires to spread rapidly, thus deforesting large areas of land. Repeated fires to maintain cleared areas would have exacerbated the loss of woody vegetation and allowed the grasslands, which responded rapidly to fire, to expand out of their pre-human habitats and become the dominant flora (McGlone, 2001, Rogers *et al.*, 2007).

This loss of forest, early in the history of human settlement in New Zealand, explains the descriptions of extensive tussock grasslands provided by early European explorers to the hinterland. The fragments of woody vegetation that remained were soon further diminished or destroyed on arrival of the European settlers in the late nineteenth century; woody vegetation was cleared to make way for sheep runs, or used as building material, for fencing or as firewood (Buchanan, 1868, Munro, 1868, Speight, 1910).

2.3 Current distribution and associations of *Podocarpus cunninghamii*

Podocarpus cunninghamii shows wide variation in its distribution and abundance across the high country. In wet, steep locations it can form extensive stands that must be reminiscent of its abundance prior to human settlement, while on dry, shallow slopes it occurs in small, isolated populations or even as individual trees. The following descriptions of the distribution of plant communities characterised by the presence of *Podocarpus cunninghamii*, which has been drawn from numerous publications and the knowledge of various individuals, will begin in the north, in the Kaikoura Ranges, before moving southwards through Canterbury and ending in Central Otago. These descriptions are not intended as a final and authoritative account of the distribution of every stand across the high country; rather, they document all of the stands that I have been able to find information on. I feel confident that the majority of stands are included here, but it is unavoidable that some

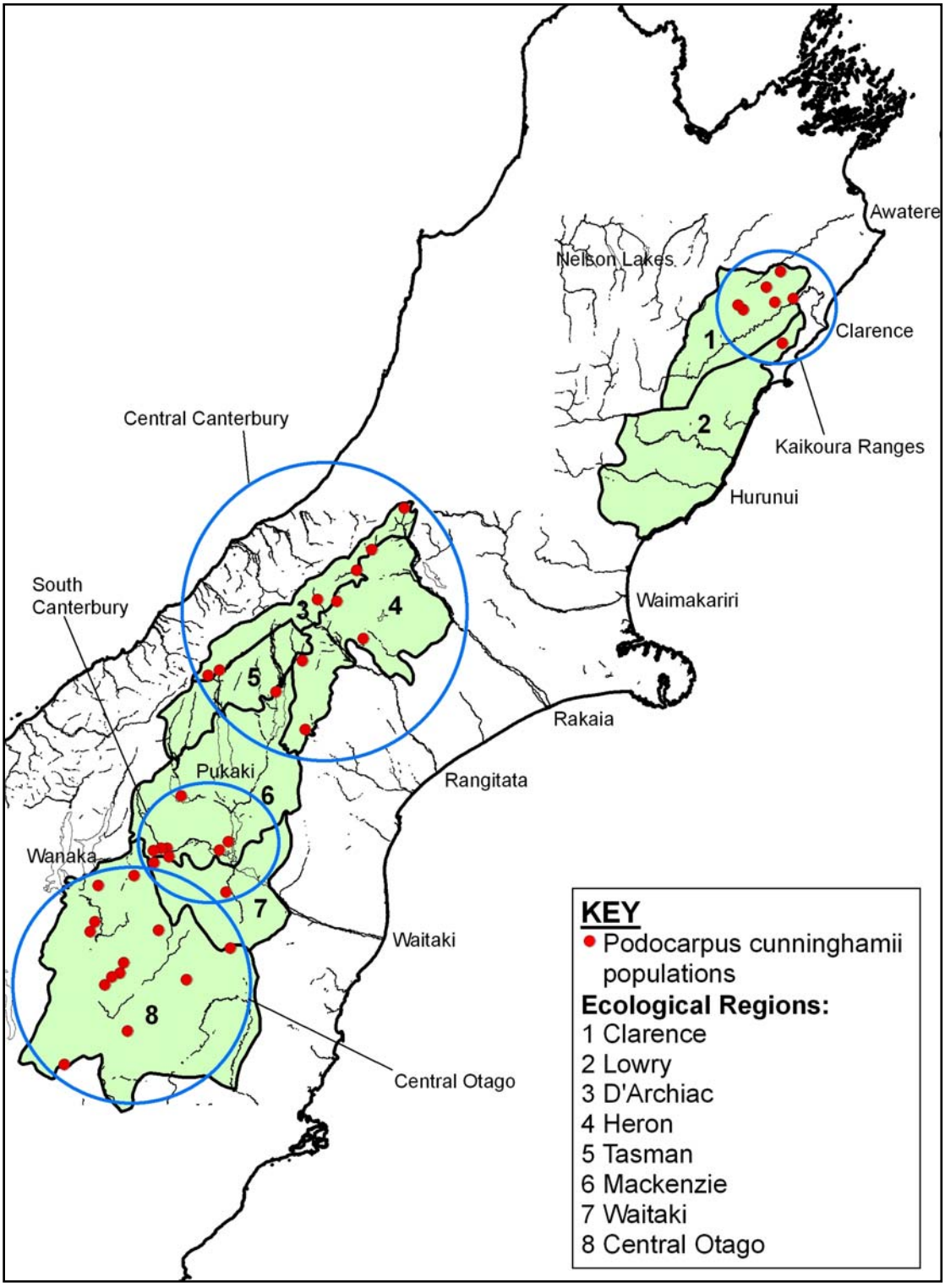

Figure 2.5 – The distribution of *Podocarpus cunninghamii* communities throughout the high country.

stands have inadvertently been missed. Eastern *Nothofagus* forests, especially those comprised of *Nothofagus solandri*, in which *Podocarpus cunninghamii* can occur, are not included in these descriptions. The descriptions are categorised firstly according to broad geographical location, and secondly more precisely, to Ecological Region (McEwan, 1978); the overall distribution is shown in Figure 2.5.

2.3.1 The Kaikoura Ranges

The Kaikoura Ranges are distinct from all other parts of the high country in that their rainfall gradient runs from east to west, rather than west to east. Greywacke is the predominant rock type (LRI, 1992).

2.3.1.1 Lowry Ecological Region

Within the steep, high precipitation (900-1,200 mm per annum) Seaward Kaikoura Range, *Podocarpus cunninghamii* forms extensive stands of tall forest, cloaking the mid-range mountain faces between 450-1,000 m altitude (Plate 2.1). *Nothofagus* becomes more important at the northern and southern ends of the range. Wardle (1971) surveyed the vegetation of the Seaward Kaikoura Range, and a summary of his descriptions of the *Podocarpus cunninghamii* vegetation association is provided here. *Podocarpus cunninghamii* is the dominant canopy tree, averaging 15 m in height, with *Griselinia littoralis, Pseudopanax crassifolius* and *Coprosma linariifolia* often forming a sub-canopy.

The shrub layer is sparse, with only *Coprosma dumosa* considered important. The ground layer is also often sparse, with occasional small herbs, such as the exotic *Mycelis muralis*, or wide-leaved species of *Uncinia* present; otherwise *Polystichum vestitum* can form dense patches. *Rubus cissoides* is the only common liana. Several species of ungulate, including red deer, are present in high numbers, and are predicted to reduce the presence of *Griselinia littoralis, Coprosma linariifolia*, *Coprosma dumosa* and *Polystichum vestitum* in the long-term. Possum use of these forests is also high, though Wardle does not discuss their potential impact on forest structure.

Plate 2.1 – Extensive stands of *Podocarpus cunninghamii* forest in the upper Hapuku River catchment, Seaward Kaikoura Range.

2.3.1.2. Clarence Ecological Region

The Inland Kaikoura Range differs markedly to the Seaward Range in terms of precipitation, receiving just 700 mm in its driest reaches (Pascoe, 1983, Williams, 1989). *Podocarpus cunninghamii* has a very fragmented distribution in the Inland Kaikoura Range, being restricted to remote and steep valleys. Charcoal deposits, pollen records, and evidence of recolonisation are all indicative of a historical cover far more extensive than that of today (McGlone and Basher, 1995, Williams, 1989).

Two forms of *Podocarpus cunninghamii* vegetation association were recorded by Williams (1989) in the Inland Kaikoura Range: *Podocarpus cunninghamii* forest and woodland, and *Podocarpus cunninghamii* scrub. Both associations occur mainly on very steep to precipitous slopes up to 1,250 m altitude. The forest and woodland association is most extensive in the southeast catchments of the Range, most notably at Muzzle and Branch Streams, while smaller, more fragmented remnants exist in the northwest catchments, particularly in Totara Stream, and the Hodder and Tone

Valleys (Plate 2.2). *Podocarpus cunninghamii* forms the canopy, reaching 12 m in height, with *Griselinia littoralis*, *Phyllocladus alpinus* and *Hoheria lyallii* forming the sub-canopy. The shrub layer, like that of the Seaward Kaikoura Range, is sparse, with *Coprosma linariifolia, Coprosma propinqua*, *Myrsine divaricata,* and *Phyllocladus alpinus* most common. The herb layer is scattered with *Polystichum vestitum, Mycelis muralis,* and fine-leaved species of *Uncinia*. The scrub association constitutes a range of shrub communities that all share the presence of emergent *Podocarpus cunninghamii*. *Discaria toumatou, Rosa rubiginosa*, *Hoheria lyallii* and *Brachyglottis monroi* are the most common sub-components. These remnant populations are often located high above the valley floors, in the mouths of hanging valleys. On very steep south facing slopes and bluffs between 1,000-1,400 m, *Phyllocladus alpinus* becomes the main sub-component with *Hoheria lyallii* and *Podocarpus nivalis*.

Plate 2.2 – Remnant stand of *Podocarpus cunninghamii* in the Tone River catchment (Muller Station), Inland Kaikoura Range.

2.3.2 Central Canterbury high country

This part of Central Canterbury, largely defined by the Heron Ecological Region but including the D'Archiac and Tasman Ecological Regions, is host to several populations of *Podocarpus cunninghamii*. These regions are all found south of the Hawdon and Puketeraki Ecological Regions, both of which are dominated by *Nothofagus* forest. Although *Podocarpus cunninghamii* can be often found growing in these *Nothofagus* forests, which inhabit the Waiau, Hurunui and Waimakariri catchments, they form a relatively minor component of the flora. Conversely, *Podocarpus cunninghamii* communities that lack *Nothofagus* are either rare or absent from these catchments. The majority of *Podocarpus cunninghamii* populations exist within the beech gap; that is the high precipitation upper catchments of the Rakaia and Rangitata Rivers, as well as in Mt Cook National Park. Some isolated remnants are also present outside of these areas. The underlying rock for the majority of this region is greywacke, with alluvial rock types common in valley bottoms and basins (LRI, 1992).

Plate 2.3 – Extensive stand of *Podocarpus cunninghamii* forest in the upper Lawrence Valley.

2.3.2.1 D'Archiac, Heron and Tasman Ecological Regions

Within the climatic diversity of these three regions, annual rainfall is highest in the west, where it can be near 7,000 mm per annum close to the Main Divide, and range from c. 2,500-5,500 mm along the Mt Cook Range (Wilson, 1976). Away from the Main Divide, such as along the flanks of Lake Tekapo, precipitation declines to 800-1,000 mm per annum.

In the Rakaia catchment, *Podocarpus cunninghamii* is recorded as being abundant above 600 m in the upper reaches of the Wilberforce River, where it forms a canopy over *Phyllocladus alpinus, Griselinia littoralis, Pseudopanax crassifolius* and *Pittosporum tenuifolium*. Other common associates include *Brachyglottis buchananii, Ozothamnus vauvilliersii* and *Anaphalioides bellidioides*. *Rubus schmidelioides* is the only liana of note, while *Polystichum vestitum* is the dominant fern along with several species of *Blechnum* (McKelvey, 1984). *Podocarpus cunninghamii* is also recorded in association with *Griselinia littoralis, Hoheria lyallii, Pseudopanax crassifolius* and *Sophora microphylla* in Lake, Jellicoe and Washbourne Streams (Burrows and Russell, 1990). Nearby in Paddle Hill Creek catchment, which feeds into the South Branch Ashburton River, remnant *Podocarpus cunninghamii* trees are found in association with *Griselinia littoralis* and *Pittosporum tenuifolium* (Burrows *et al.*, 1993).

In the Rangitata catchment, *Podocarpus cunninghamii* is present in the upper reaches of the Lawrence River, forming a canopy over *Phyllocladus alpinus, Griselinia littoralis, Podocarpus nivalis,* as well as various *Hebe* and *Coprosma* species (Plate 2.3). *Podocarpus cunninghamii* is also recorded as forming a remnant forest/scrub association with *Kunzea ericoides* on the cone and talus systems north of Lake Clearwater, at the southern tip of the Dogs Range, opposite Mt Guy. (Harrington *et al.*, 1986). Annual rainfall in the upper Lawrence is approximately 1,500 mm, which declines to 800-1,000 mm around Mt Guy (Ryan, 1987).

Further south, *Podocarpus cunninghamii* is found along the Two Thumb Range. In the headwaters of Lake Tekapo, in the streams along the true left of the Macaulay River, *Podocarpus cunninghamii* forms shrub communities with *Aristotelia fruticosa* and numerous *Coprosma* species. At the southern extent of the same Range,

Podocarpus cunninghamii forms a significant forest stand with *Pseudopanax crassifolius* and *Griselinia littoralis* (Plate 2.4). Just east of here, in the South Opuha River, several specimens of *Podocarpus cunninghamii* are recorded as being present amongst remnant stands of *Pseudopanax crassifolius, Griselinia littoralis* and *Pittosporum tenuifolium* (Harrington *et al.*, 1986). Annual rainfall is high in the northwest of the Range, reaching 2,000 mm per annum. This declines rapidly further south, to 1,000 mm (Ryan, 1987).

Plate 2.4 – *Podocarpus cunninghamii* forest at the southern end of the Two Thumb Range.

Several stands of *Podocarpus cunninghamii* forest are present within Aoraki/Mt Cook National Park (MCNP), on the Liebig, Mt Cook and Sealy Ranges (Plate 2.5). Here *Podocarpus cunninghamii* emerges above a canopy of predominantly *Griselinia littoralis* and *Phyllocladus alpinus*, with *Pseudopanax colensoi* var. *ternatus, Hoheria lyallii* and *Dracophyllum longifolium* also common (Wilson, 1976). Outside of MCNP, to the southeast, within the Tasman Ecological Region, small remnant stands of *Podocarpus cunninghamii* occupy the faces of the Hall Range, below Mistake

Peak, overlooking the head of Lake Tekapo (D. Scott, pers. comm.), and in the upper reaches of the Cass River (D.A. Norton, pers. comm.).

Plate 2.5 – A stand of *Podocarpus cunninghamii* forest on the Sealy Range, near Mt Cook Village.

2.3.3 South Canterbury high country

The south Canterbury high country is a dramatic and extremely varied landscape, covering the Mackenzie and Waitaki Ecological Regions. Close to the Main Divide rainfall can reach 2,000 mm per annum, while further east, along the flanks of Lakes Pukaki and Ohau, precipitation declines to 800-1,000 mm per annum. Further east again, at the southern tip of the Diadem Range, along the Ahuriri River, and along the Hawkdun Range, the climate becomes semi-arid, and annual rainfall can drop to below 600 mm. Greywacke is the most common underlying rock type on the mountain faces, with alluvial deposits forming the basins. The greywacke begins to intergrade with schist south of the Ahuriri River (LRI, 1992).

2.3.3.1 Mackenzie and Waitaki Ecological Regions

An extensive stand of *Podocarpus cunninghamii* is present on the boulderfields of the southwest face of Mt Ben Ohau, overlooking Lake Ohau (Plate 2.6). This particular stand is regarded as being the best example of a regenerating stand of *Podocarpus cunninghamii* in the Ben Ohau Ecological District (Espie *et al.*, 1984), and may provide the most accurate picture of the dry *Podocarpus cunninghamii* forests that once covered the wider area (Molloy *et al.*, 1976). Three lonesome individuals are also recorded in Lockharts Stream, east of the Dalgety Range.

Plate 2.6 – *Podocarpus cunninghamii* on the southwest face of Mt Ben Ohau.

Several stands are present in the drier areas of the Mackenzie Region, particularly on the hill slopes in and around the Ahuriri Valley, at the southern end of the Diadem Range (Plate 2.7). These form associations with *Podocarpus nivalis* and *Phyllocladus alpinus*, and occur mainly on boulderfields. *Podocarpus cunninghamii* populations in association with various *Coprosma* species are also recorded on the boulderfields within Totara and Coal Creeks, on the Benmore Range (Espie *et al.*, 1984). A small stand of *Podocarpus cunninghamii* is also present further east, down the Waitaki

River system, perched high on a south facing ledge in a deep gorge of Clear Stream (D.A. Norton, pers. comm.); annual rainfall here is less than 600 mm.

Plate 2.7 – *Podocarpus cunninghamii* remnant at the southern end of the Diadem Range (Birdwood Station).

2.3.4 Central Otago

The Central Otago region is home to New Zealand's most continental climate, with annual rainfall levels dropping to as low as 400-500 mm in the driest places (De Lisle and Browne, 1968). The region is also distinct from the areas further north as the rock underlying the mountain ranges is almost entirely schist rather than greywacke (LRI, 1992).

2.3.4.1 Central Otago Ecological Region

There are numerous small population of *Podocarpus cunninghamii* within the Central Otago Ecological Region, many comprised of just a handful of trees. As Wardle (2001a) notes in his descriptions of the flora in the upper Clutha district, the drier mountains, down valley from the more extensive, predominantly *Nothofagus* forest

areas, exist scattered and small *Podocarpus cunninghamii* communities. These typically include species such as *Podocarpus nivalis*, *Phyllocladus alpinus*, *Dracophyllum longifolium*, *Olearia nummularifolia*, *Olearia cymbifolia*, *Brachglottis cassinioides* and *Hebe subalpina*.

Plate 2.8 – Remnant stand of *Podocarpus cunninghamii* mixed with *Kunzea ericoides* at Alfern Creek Conservation Area.

Podocarpus cunninghamii communities are present on the east side of the Lindis Pass Road, at the base of McLays Creek and on the steep, south facing slopes of Dip Creek, where it associates with *Olearia odorata*, *Coprosma propinqua* and *Discaria toumatou*. In the gorge area of Hospital Creek, near Lake Hawea, scattered *Podocarpus cunninghamii* trees form communities with *Olearia avicenniaefolia*, *Coprosma propinqua* and *Helichrysum aggregatum*. Two remnant populations also occur further south, at Alfern Creek and the Lochar Burn, on the Pisa Range (Plate 2.8). These sites are rich with *Kunzea ericoides*, which shelter smaller numbers of *Podocarpus cunninghamii*, *Podocarpus nivalis*, *Phyllocladus alpinus*, *Dracophyllum longifolium*, *Corokia cotoneaster* and *Coprosma* species. Further east, at the northern and southern extents of the Dunstan Mountains, are additional *Podocarpus cunninghamii* populations. At Shepherd's Creek, *Podocarpus cunninghamii* has colonised rocky outcrops and rockfields with *Corokia cotoneaster*, *Griselinia*

littoralis and *Phyllocladus alpinus*; at Scotts Creek, the population is again confined to a rocky fire refuge, and here grows with *Phyllocladus alpinus, Dracophyllum longifolium* and *Podocarpus nivalis* (Ward *et al.*, 1994). *Podocarpus cunninghamii* is also recorded as being present in the gorge of Near Undaunted Creek, on the south face of the Ida Range (Grove, 1994).

2.4 Summary

The predominant vegetation types of the high country have not been constant through time. At the end of the last glacial maximum grasslands covered much of the landscape. At around 13-10,000- years BP, as the climate warmed, these were slowly succeeded by *Coprosma* type shrublands or *Phyllocladus* scrub below tree line. Eventually, about 10-8,000 years BP, as temperatures continued to rise, podocarp forest expanded into these woody communities and became the predominant vegetation type. Approximately 7-6,000 years BP the climate began to cool again, allowing *Nothofagus* to expand its range and replace podocarp forest along the vast majority of the axial ranges. *Podocarpus cunninghamii* forest remained the dominant forest type in the central part of the South Island, where *Nothofagus* was still largely absent. The absence of *Nothofagus* here most likely reflects its slow dispersal from refugia to the north and south of this region since end of the last glacial maximum. Moving eastwards from the Main Divide into the drylands, *Podocarpus cunninghamii* communities remained the dominant woody community below tree line and above the valley bottoms. For the most part, shrublands and tussock grasslands would have been confined to valley bottoms and alpine areas, respectively. This state is most likely what the first Polynesians to New Zealand would have encountered approximately 800 years ago. Vast swathes of the high country were deforested during the early stages of Polynesian settlement. This deforestation was greatest in the eastern dryland high country, and explains the predominantly grassland environment that early European explorers and pastoralists discovered. Pastoral farming has extended and maintained these grasslands over the last 150 years.

The large scale deforestation and conversion of the high country to grassland has severely depleted the former, pre-human range of *Podocarpus cunninghamii* communities. In the wetter valleys close to the Main Divide, such as in the

headwaters of the Rakaia and Rangitata catchments, extensive tracts of tall *Podocarpus cunninghamii* forest can still be found. Species such as *Griselinia littoralis*, *Pseudopanax crassifolius* and *Coprosma linariifolia* are common and form a continuous canopy or sub-canopy, above which project emergent *Podocarpus cunninghamii*, at times frequent enough to form a canopy. However, as one moves eastwards, the remaining populations become increasingly scarce, small and isolated. These short statured, predominantly scrub-like communities cling to fire refugia and appear highly degraded. Species of *Coprosma* are common, as is the invasive *Rosa rubiginosa*.

3 The ecology of *Podocarpus cunninghamii* in the high country

3.1 Introduction

Prior to human settlement the eastern South Island high country was covered in woody vegetation below the alpine tree line (McGlone, 1989). Charcoal deposits, log remains and palynological records all indicate that *Podocarpus cunninghamii* was a dominant tree species within these historical woody communities, especially in areas where *Nothofagus* was absent (e.g. Buchanan, 1868, McGlone, 2001, McGlone and Basher, 1995, Molloy *et al.*, 1963, Munro, 1868). However, human activities, especially burning by both Polynesians and Europeans, and subsequent European agricultural expansion, has led to a marked decline in the cover of woody vegetation and a concomitant increase in grassland cover (McGlone, 2001, McGlone, 2004). Despite this widespread destruction, remnants of *Podocarpus cunninghamii* dominated plant communities are still present through the high country.

A clear rainfall gradient occurs across the high country; areas immediately east of the Main Divide, such as Aoraki/Mt Cook National Park, have high annual precipitation (2,500-5,000 mm); but further east in the inter-montane basins, such as the Mackenzie Basin and Central Otago, annual rainfall is considerably less (<600 mm); the Kaikoura Ranges are anomalous in that their rainfall gradient runs from west to east. In the areas of high precipitation, such as in the headwaters of the Wilberforce River and the Seaward Kaikoura Range, extensive tall forest stands still remain (McKelvey, 1984, Wardle, 1971). However, in the drier areas *Podocarpus cunninghamii* dominated vegetation has been reduced to a very few small and isolated remnants that exist within a highly modified, predominantly grassland environment (Norton, 2006, Walker *et al.*, 2004b). These remnants are the last remaining examples of the dryland high country's pre-human flora, but are often highly modified, due to their small size and susceptibility to edge effects, as well as their long history of fire and grazing disturbance (Walker *et al.*, 2004a).

Very little is known about the ecology of *Podocarpus cunninghamii* communities within the high country – they are among the least understood and least studied ecological systems in New Zealand (Rogers *et al.*, 2005, Walker *et al.*, 2003). As a result, little is known of the vegetation communities *Podocarpus cunninghamii* forms and how community composition is affected by environmental gradients. Furthermore, there is little knowledge of *Podocarpus cunninghamii* stand structures and regeneration patterns in the high country. Such information is important for the ongoing management and future restoration of these communities. The objectives of this research are to describe the contemporary flora of *Podocarpus cunninghamii* vegetation communities across the eastern South Island high country, to investigate how or whether vegetation patterns show a geographical distribution, and to determine what environmental factors drive these patterns. In addition, this research will investigate the population dynamics of *Podocarpus cunninghamii* stands across the high country, using dendrochronological methods, to provide an insight into the history and trajectory of these stands, and to elucidate how disturbance regimes may affect current and future *Podocarpus cunninghamii* age and size structures. The implications of the research for restoration will then be discussed.

3.2 Methods

3.2.1 Study sites

The study focussed on the South Island high country: the mountains and basins in the rain-shadow of the Southern Alps, distributed through Marlborough, Canterbury and Otago (Swaffield and Hughey, 2001). This area comprises the Clarence, Lowry, Heron, D'Archiac, Tasman, Mackenzie, Waitaki and Central Otago Ecological Regions (McEwan, 1978; Figure 2.1).

Individual sites containing *Podocarpus cunninghamii* dominated communities were identified from survey reports of the Protected Natural Areas Programme (PNAP) covering the Heron, Mackenzie and Central Otago Ecological Regions (Espie *et al.*, 1984, Harrington *et al.*, 1986, Ward *et al.*, 1994), the Vegetation of Mt Cook National Park (Wilson, 1976), and three journal publications (McGlone and Basher, 1995, Wardle, 1971, Williams, 1989). Stands were selected for study only if they were large enough to constitute woodland; therefore those sites that were recorded in the

PNAP reports as having only one or a few remnant trees of *Podocarpus cunninghamii* were not included. One additional site, not identified from the above sources, was included based on local knowledge; this was Godley Peaks (Mackenzie Ecological Region; D. Scott, pers. comm.). The 12 selected sites are listed in Table 3.1, along with site codes, and are mapped in Figure 3.1. Six of the 12 sites were selected for investigation of population dynamics; stand characteristics are given in Table 3.2.

3.2.2 Data collection

3.2.2.1 Vegetation surveys

Vegetation surveys of *Podocarpus cunninghamii* dominated vascular plant communities were undertaken using unbounded plots of approximately 95 m^2 (Hurst and Allen, 2007); this area was sometimes reduced when the vegetation patch was marginally smaller than the desired plot size. Sample plots were placed randomly along transect lines at 50 m altitudinal increments (e.g. a transect line was run at 800 m, 850 m, 900 m, etc) with each plot at least 20 m apart at each altitude. However, due to the very patchy nature of some of the remnants, i.e. small discrete stands of *Podocarpus cunninghamii* scattered within a rock field, it was not possible to place each plot at a specific altitude or distance apart. In such cases the closest patch of *Podocarpus cunninghamii* vegetation to the transect was sampled. The number of altitudes sampled was dependent on the altitudinal range of the site. The number of plots per site was similarly limited by extent (Table 3.1). Where large, continuous areas of forest were sampled, plots were located at least 10 m away from the edge of the stand in an attempt to avoid recording edge vegetation. Within the more scattered and patchy stands it was often impossible to avoid sampling edge vegetation, as the size of the patches did not allow for any non-edge vegetation.

The following data were recorded for each plot:

1) Physical characteristics:

   GPS coordinates, altitude, aspect, slope, physiography (ridge, face, gully, terrace), surface cover (% rock, bare ground, litter, vegetation, non-vascular vegetation), average top height and canopy cover.

2) Vegetation cover according to height strata:

Three height strata were used; stratum one (>3 m), stratum two (0.5-3 m), and stratum three (0-0.5 m). Any species with foliage present in more than one stratum was recorded as being in each stratum it was found. Plant percentage cover within each stratum was estimated using a modified Braun-Blaunquet scale (Fuller and Conard, 1965):

1. <1% cover
2. 1-5% cover
3. 6-10% cover
4. 11-25% cover
5. 26-50% cover
6. 51-75% cover
7. 76-100% cover

Samples were collected of any species that was unidentifiable in the field and subsequently taken to a botanist for identification. Samples were pressed and stored for future reference.

3) Soil properties:

A soil sample to 10-15 cm depth was collected from each plot using a 2.5 cm diameter soil borer. Samples were sieved to 2 mm and left to air dry. Equal amounts of soil were then taken from each plot sample and mixed to provide a 100 g site sample; this formed a representative sample of the soil at a site. The soil then sent to Hill Laboratories Ltd (a commercial analytical laboratory) for analysis of pH, Olsen phosphorous (P), total nitrogen (N) and total carbon (C).

Additional coarse scale data were also derived for each site and used in the analysis. The following data were extracted from the Land Environments of New Zealand (LENZ) 25 m^2 database: mean annual soil moisture deficit, vapour pressure deficit and solar radiation, mean annual temperature, and the minimum temperature of the coldest month.

Table 3.1 – The 12 study sites with their location, Ecological Region and District. The area sampled is the total patch of continuous forest sampled. For DR, GP, LS, LI and PR each plot often constituted a patch, thus the area sampled for these sites is the sum of multiple patches. * indicate sites where investigation of population dynamics was also conducted.

Site Name	**Ecological Region**	**Ecological District**	**Locality**	**Size of remnant** (ha)	**Number of plots**
Tone River 1 (TR)	Clarence	Dillon	42° 05' S, 173° 25' E	0.6	3
Tone River 2 (TR)			42° 04' S, 173° 23' E	3.2	6
Upper Hapuku Valley (UH)*	Lowry	Hundalee	42° 14' S, 173° 40' E	14.6	12
Upper Lawrence Valley 1 (UL)*	Heron	Arrowsmith	43° 24' S, 170° 54' E	2.4	5
Upper Lawrence Valley 2 (UL)			43° 24' S, 170° 53' E	16.3	12
Dogs Range (DR)	Heron	Arrowsmith	43° 34' S, 171° 03' E	1.4	8
Mt Dobson (MD)*	Heron	Two Thumb	43° 58' S, 170° 41' E	2.1	6
Aoraki/Mt Cook National Park (MC)*	D'Archiac	Mt Cook	43° 41' S, 170° 09' E	7.7	12
			43° 43' S, 170° 05' E	2.9	3
Godley Peaks (GP)	Tasman	Godley	43° 48' S, 170° 30' E	1.6	7
Mt Ben Ohau (MB)*	Mackenzie	Ben Ohau	44° 15' S, 169° 53' E	10.5	15
Birdwood Station (BS)*	Mackenzie	Ahuriri	44° 29' S, 169° 47' E	8.1	15
Longslip Station (LS)	Mackenzie	Ahuriri	44° 29' S, 169° 44' E	3.5	9
Lindis (LI)	Mackenzie	Ahuriri	44° 30' S, 169° 41' E	3.3	8
Pisa Range 1 (PR)	Central Otago	Pisa	44° 49' S, 169° 18' E	0.3	2
Pisa Range 2 (PR)			44° 51' S, 169° 16' E	2.9	7

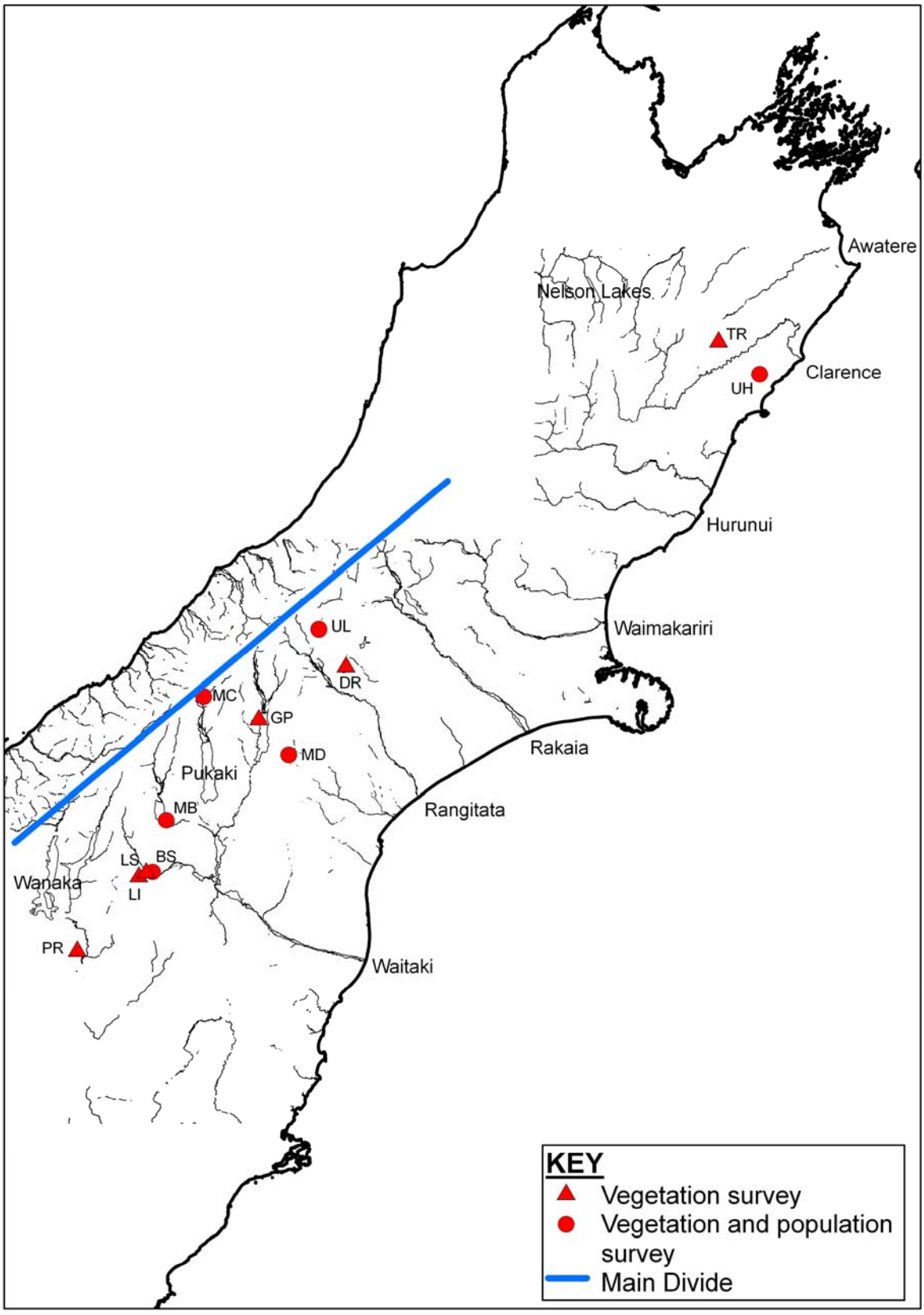

Figure 3.1 – Locations of the 12 study sites.

3.2.2.2 Population dynamics

Rectangular plots of 0.05-0.4 ha were established within six of the study sites (Figure 3.1). The area of each plot was dependent on the spacing of *Podocarpus cunninghamii* tress, with the objective being to sample at least 50 trees with a diameter at breast height (dbh; 1.4 m) ≥ 5 cm. A single increment core was extracted at 50-140 cm above ground level from all trees with a dbh ≥5 cm. In addition, ten sampled trees were randomly selected from each of sites Birdwood Station, Mt Ben Ohau and Mt Cook National Park, and three cores extracted from each; these were used to calculate an error rate associated with the age estimates. The height at which an increment core was extracted was affected by forked stems and the need to avoid bulges, crooks and fused stems. Where trees had multiple stems that originated below 1.4 m, the two stems with the greatest dbh were selected, measured, and an increment core extracted from both. The dbh of all trees ≥5 cm dbh was recorded, and the heights of all saplings and trees were estimated using a Suunto clinometer.

Seedlings, saplings and trees encountered were classified as such according to the following criteria (modified from Veblen & Stewart (1982)):

- Established seedling: >50 cm, ≤1.4 m height
- Sapling: >1.4 m height, <5 cm dbh
- Tree >1.4 m height, ≥5 cm dbh

In this survey the only seedlings of interest were those considered to have successfully established. As such, seedlings less than 50 cm height were considered ephemeral and thus not enumerated.

Cores were stored in plastic straws prior to arrival in the laboratory. Once in the lab they were left to air dry for two weeks before being mounted onto grooved wooden boards, transverse surface upwards. After 24 hours each core was sanded, first with a 120 grade belt sander, followed by a 280 grade orbital sander. Cores were examined under a binocular dissecting microscope and the number of rings counted (Stokes and Smiley, 1968).

Where the chronological centre of the core was missed, or the core was rotten, it was necessary to estimate the age of the core. This was achieved using Equation 3.1.

$$A = (r - p) / (d / 20) + N \qquad \text{(Equation 3.1)}$$

Where A is age, r is the geometric radius, p is the length of the partial core, d is the width of the innermost 20 rings, and N is the number of rings counted from the partial core (Norton *et al.*, 1987).

3.2.3 Statistical analysis

3.2.3.1 Vegetation surveys

Two forms of analysis were conducted: classification and ordination. The aim of the classification was to group the different vegetation types into species-based classes. The aim of the ordination was to identify how vascular plant species are distributed across the sites and plots according to a range of environmental variables.

Prior to analysis the vegetation data for each plot, split across three height strata, was combined into a single value per species. This was achieved by taking the $\log_{10}$ of the median height of each height stratum (the median of the >3 m stratum was set at 7 m) and multiplying by the median percent cover class of that species in that stratum (Norton and Leathwick, 1990). Taking $\log_{10}$ of any number less than one produces negative values; in reality it is impossible to have a negative cover value. As such, all negative values were converted into positive numbers of the same value (e.g. convert -0.46 to 0.46) by taking the square root of the square.

3.2.3.1.1 Classification

The vegetation data were separated into different classes by two-way indicator species analyis (TWINSPAN) using WinTWINS (TWINSPAN for Windows version 2.3 (Hill and Šmilauer, 2005)). TWINSPAN works by conducting repeated ordinations and divisions of the plot and species data, thereby producing a dichotomous classification tree. Several cut levels (both the number of cut levels and their values) were evaluated, as well as the effects of omitting rarely occurring species. The results from these were viewed in light of field observations of community patterns, and in the end the default programme values were selected for final analysis: five pseudo-species cut

levels with values of 0, 2, 5, 10 and 20. All recorded species, irrespective of their rareness, were included in the analysis.

The species-based classes were named following the system proposed by Atkinson (1985) and modified by Grüner (2003). In this way the two most common woody species (≥20% cover) of tallest stature are selected; their names form the basis of the community name together with their form, i.e. forest, scrubland or woodland. Species are then placed in rounded () or square [] parentheses if their abundance is below 20% or 10% respectively. If two classes have the same two most common species, the third most common species is also included in the name.

The Shannon diversity index (Shannon, 1948, Shannon and Weaver, 1949) was calculated for each class using the vegan package (version 1.15.4) (Oksanen *et al.*, 2009) in R 2.10.1 (R Development Core Team, 2009) and compared between classes using ANOVA. To measure how 'invaded' each class was, counts of the number of exotic species present within each plot within each class were analysed using generalised linear models with a Poisson family error distribution. The environmental attributes and structural characteristics of each class were also calculated. These class values were compared by ANOVA, to gain insight into how these classes differ and what variables might be driving these differences.

3.2.3.1.2 Ordination

To elucidate how sites and species vary along the measured environmental gradients, the vegetation data was first analysed using principal components analysis (PCA) on Hellinger transformed species data. Hellinger transformations maintain Euclidean distances between plots but provides resilience in the analysis against multiple zeros; this avoids the horseshoe effect that can compromise classical correspondence analysis or PCA on untransformed data (Legendre and Gallagher, 2001). Each environmental variable was then tested for any relationship with PCA axes one and two using Pearson's product-moment correlation. Those environmental variables found to be significant ($p<0.05$) were retained for use in canonical redundancy analysis (RDA).

The Hellinger transformed vegetation data was then analysed in conjunction with the selected environmental variables by RDA. RDA constrains the data held in the species data matrix by multiple linear regressions to the environmental data matrix (Legendre and Legendre, 1998) and allows visual interpretation of how the sites vary along the environmental gradients. RDA was selected over the related canonical correspondence analysis (CCA) because RDA uses the Euclidean distance rather than the Chi-squared distance, as CCA does; the Chi-squared distance gives heavier weighting to rare species (Legendre and Gallagher, 2001), which was not considered desirable in the present case. All analyses were conducted in R 2.10.1, using the vegan package.

3.2.3.2 Population dynamics

3.2.3.2.1 Age and size class distributions

To gain an understanding of the current population structures of the different stands, age and size class distributions were constructed. For trees that had two stems measured, the dbh and height measurements were averaged. Stand basal area and stem density were also calculated.

Linear regressions were conducted per site on age against dbh and age against height data; data were log-transformed where necessary. Stand basal areas were estimated using dbh values. The basal area of trees that had two stems measured was estimated by calculating and summing the individual values for the two stems.

Using the three cores that were extracted from ten trees at each of Birdwood Station, Mt Ben Ohau and Mt Cook National Park, two estimates of age error by dbh class were calculated. The first was a measure of the percent underestimate of age of an increment core within a given dbh class, and was calculated using Equation 3.2.

$$U = ((A_o - A_y) / A_o) \times 100 \qquad \text{(Equation 3.2)}$$

Where U is the percent age underestimate, A_o is the age of the oldest core and A_y is the age of the youngest core. This was calculated for dbh classes 5-10, 10-20, 20-30 and 30-50 cm. This provided a mean percent underestimate per size class. All age counts are likely to be minimum estimates of tree age (see below); as such, of the

three cores taken from a single tree, the one with the most rings was considered to represent the best estimate of minimum age.

The second error estimate was that of age variation within a given dbh class. This was calculated by taking the oldest of the three cores per tree and placing it within the relevant 5-10, 10-20, 20-30 or 30-50 cm dbh class. This provided a mean age per size class, from which the standard error was then calculated. Given the uncertainty of the age-height relationship, an error rate was not calculated for age by height class. All analyses and graphical operations were conducted using R 2.10.1.

3.2.3.2.2 Classification

Cluster analysis was conducted on the age-class distributions to objectively identify differences between the distributions of the six sites. A dissimilarity matrix was first constructed using the Jaccard index. Clusters were then identified and assigned to classes through hierarchical agglomerative clustering using the unweighted pair group method with arithmetic means (UPGMA) (Legendre and Legendre, 1998). Analysis was conducted in R 2.10.1, using the vegan package.

3.2.3.2.3 Methodological limitations

There are several limitations involved with the dendrochronological approach to estimating population age structure (Norton and Ogden, 1987, Ogden, 1985). The age-diameter relationship suffers from the main issue that extrapolations of age from stem diameter are subject to the extreme variance that can occur for stem diameters within an age class, or vice versa (Ogden, 1985); as such, "the use of size as a measure of age is only justified if the relationship has been formally proved by the use of some independent accurate measure of age" (Harper, 1977). In addition, there are problems associated with counting tree rings. Such problems can arise from rotten tree cores or simply from the borer missing the centre of the tree; both result in partial cores, meaning that the full ring chronology cannot be analysed (Dunwiddie, 1979, Norton *et al.*, 1987). Further problems can arise from anomalous rings, which can be split into three different kinds: false rings, frost rings, and missing rings (Norton and Ogden, 1987). False rings are generated as a result of periods of adverse environmental conditions during the growing season, e.g. a short summer drought, which causes radial growth to cease for a period, before restarting again. Frost rings,

which are a form of false ring, can occur when cells suffer frost damage, and can be common in subalpine trees. Missing rings can arise when conditions are so severe that radial increment is localised to only certain parts of the tree or does not occur at all (Norton, 1986). The issue of false and missing rings is known to be particularly prevalent in *Podocarpus cunninghamii* (Bell and Bell, 1959, Scott, 1972).

There are several ways of attempting to control for the problems associated with dendrochronological data. The problem of incomplete or partial cores can be overcome to some extent by tracing the arc of the innermost ring of a core and fitting a full circle using a compass, thus identifying the missing radius of a core. The number of rings missed within this can then be estimated by dividing the width of the missing radius by the width of the innermost twenty rings (Duncan, 1989, Norton *et al.*, 1987); this is the method outlined above (Equation 3.1).

The problems of false and missing rings are more difficult. Bell & Bell (1959) recommend that studies of totara (both *Podocarpus totara* and *Podocarpus cunninghamii*) tree rings use complete cross-sections rather than increment cores. This was not practicable in this instance as many of the *Podocarpus cunninghamii* populations in question are within public conservation land, while those occurring on private land are so small that felling even a single tree would be highly irresponsible. Another method has been suggested that, while not overcoming the problems of false and missing rings, greatly limits their degree of error. The method is simply to extract cores from the longest radii of the stem (Duncan, 1989). This is the method that was used in the present study. In addition, multiple cores were taken from a selection of trees, which enabled an error rate to be calculated and applied to the age estimates. It should be noted that due to the frequency of missing rings, especially those arising from "ring wedging" (Dunwiddie, 1979, Norton *et al.*, 1987), all ring counts from increment cores are likely to represent minimum rather than maximum age estimates. Therefore, to be explicitly clear, all ages recorded and discussed in this chapter are estimates of tree ages based on ring counts, which are considered to be underestimates; they are not calendar years. Furthermore, the ages recorded do not take into account the length of time it takes for a *Podocarpus cunninghamii* seedling to reach 1.4 m height; this data is absent from the literature and it was not considered ethical to collect seedlings for aging from the study sites because of their rarity.

Table 3.2 – Stand characteristics of *Podocarpus cunninghamii* at the six population dynamics study sites.

Site	**Altitude** (m)	**Plot size** (ha)	**Stem density** (stems·ha^{-1})	**Basal area** (m^2·ha^{-1})	**Average height** (m)
UH	850	0.2	250	35.29	9.90
UL	850	0.2	250	44.65	12.25
MC	800	0.4	125	6.17	6.97
MD	850	0.21	238	14.13	6.05
MB	750	0.12	416	10.02	4.04
BS	700	0.05	1000	6.27	3.68

Table 3.3 – LENZ environmental data for each site. All figures are annual means.

Site	**Soil water deficit** (mm)	**Vapour pressure deficit** (kPa)	**Mean temp.** (°C)	**Min. temp.** (°C)	**Solar radiation** (MJ·m^{-2}·d^{-1})
TR	130.00	0.45	7.28	-3.26	14.80
UH	0.00	0.50	8.09	-1.53	14.60
UL	0.00	0.44	8.00	-1.95	13.99
DR	14.75	0.43	7.53	-2.10	13.90
MD	0.00	0.38	8.08	-2.30	13.40
MC	0.00	0.39	8.03	-2.30	13.85
GP	85.71	0.48	8.17	-2.26	14.40
MB	30.13	0.43	8.20	-2.45	13.89
BS	98.73	0.43	8.15	-3.07	13.79
LS	86.22	0.42	7.94	-3.04	13.80
LI	83.25	0.42	7.93	-3.05	13.80
PR	98.00	0.40	7.56	-3.59	13.83

3.3 Results

3.3.1 Study sites

Six of the 12 study sites, namely Pisa Range, Birdwood Station, Longslip Station, Mt Cook National Park, Upper Lawrence and Tone River, comprised more than one distinct stand of *Podocarpus cunninghamii* forest. For all sites apart from Upper Lawrence and Tone River, each population within a site fell under the same tenure or management type. Upper Lawrence and Tone River are exceptions in that of the two populations sampled at each, one was situated on farmland while the other was situated on DOC administered public conservation estate. A brief description of each site follows, and Table 3.3 summarises the environmental data obtained from LENZ.

3.3.1.1 Tone River (TR)

Two sites were visited within the Tone River catchment. The larger of the sites, situated within the Ka Whata Tu O Rakihouia Conservation Park, lies on the true left of the Tone River, approximately 1 km south of the mouth of the Nesbit Stream; no *Podocarpus cunninghamii* was seen on the true right of the river. The second site, located on Muller Station, is much smaller and occurs within the upper reaches of a deeply incised valley to the east of Mt Muller. Both sites are steep (35-40°) and have a high ground cover of rock.

The larger site has a more open structure, with individuals of *Podocarpus cunninghamii* scattered throughout a diverse and dense tree/shrubland (Plate 3.1). *Hebe traversii*, *Hoheria lyallii*, *Pittosporum tenuifolium* and *Brachyglottis monroi* are all abundant. *Phyllocladus alpinus*, *Olearia nummulariifolia* and *Coprosma linariifolia* are also common. Prevalent exotic species include *Rosa rubiginosa*, *Ribes uva-crispa* and *Hieracium lepidulum*. Large standing dead specimens of *Podocarpus cunninghamii* occur frequently (up to 10 m tall), and several of the larger live trees show advanced stages of crown dieback. Regeneration of *Podocarpus cunninghamii* is apparent throughout the site.

The smaller site has a more typical tall forest structure with a closed *Podocarpus cunninghamii* canopy (10 m tall; Plate 2.2). Large dead trees are again common. The shrub element is relatively sparse, apart from where it proliferates within canopy gaps

along with saplings of *Podocarpus cunninghamii*. *Pseudopanax colensoi* var. *ternatus* has a very limited occurrence and only in seedling form. Goats and possums were seen in the area.

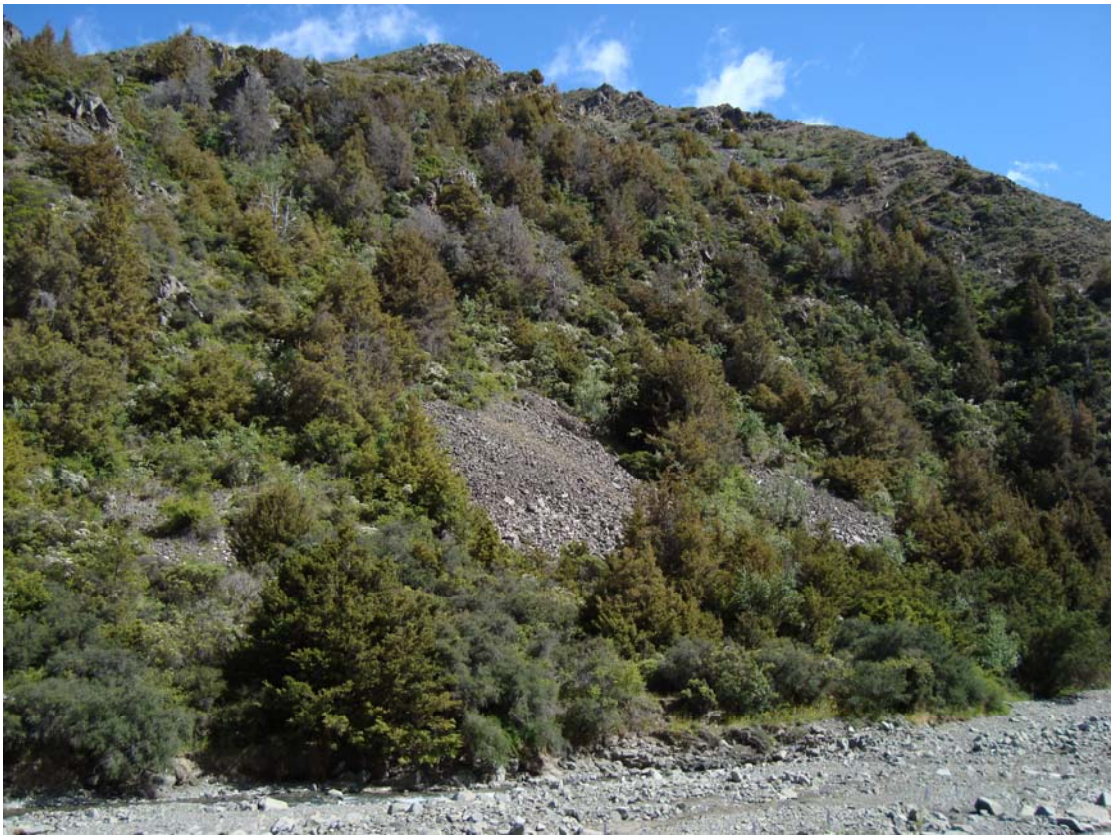

Plate 3.1 – The larger of the two sites sampled at Tone River.

3.3.1.2 Upper Hapuku (UH)

The site studied is located within a series of steep valleys cloaked in *Podocarpus cunninghamii* dominated forest. Extensive stands of large *Podocarpus cunninghamii* trees (15-20 m tall) dominate the vegetation (Plates 2.1 and 3.2). Given the abundance and size of *Podocarpus cunninghamii* at this site, these stands are considered to represent old growth forest. *Griselinia littoralis*, *Pseudopanax crassifolius* and *Coprosma linariifolia* occur frequently, though not in great enough numbers to form a contiguous sub-canopy. Shrubs such as *Coprosma propinqua* and *Hebe traversii* are common; together with the aforementioned species they form a two-tiered understorey. Herbivore damage to all sub-canopy species is clear, with seedlings, saplings and epicormic shoots all showing evidence of browsing; it is probable that this is the reason for the sparse angiosperm canopy. This area is popular

amongst hunters. A pig nest and deer wallow was found during the visit; possums were also seen.

Plate 3.2 – Mature *Podocarpus cunninghamii* trees project above shrub species at Upper Hapuku.

3.3.1.3 Upper Lawrence (UL)

Located approximately 7-8 km up the Lawrence River from the confluence with the Clyde River, this site represents a relatively extensive area of *Podocarpus cunninghamii* dominated vegetation. Large *Podocarpus cunninghamii* trees (15-20 m tall) form an emergent layer above an angiosperm canopy dominated by *Griselinia littoralis* (3-5 m tall; Plates 2.3 and 3.3). Other common trees and shrubs were *Phyllocladus alpinus, Pseudopanax crassifolius, Coprosma linariifolia, Coprosma propinqua* and *Myrsine divaricata*. The sheer size and abundance of *Podocarpus cunninghamii* trees at this site, together with the intact broadleaf canopy, suggests that while the historical forest area has suffered a considerable reduction, the remaining stands are relatively intact and representative of old growth forest.

Plate 3.3 – Mature *Podocarpus cunninghamii* trees project above the angiosperm canopy and shrub layer at Upper Lawrence.

Podocarpus cunninghamii occurs on both sides of the river; the vast majority lie on the river's true right, forming part of the Hakatere Conservation Park; the remaining stand lies on the true left of the river, and is part of Erewhon Station.

Dealing with the larger area first, several disconnected stands occur both on the lower faces with a relatively gentle slope (approximately 25°) and higher up on extremely steep rock outcrops. All stands occur on stable rock fields and are separated by unvegetated sloping rock fields. The largest stand present was sampled (Table 3.1).

The smaller area, situated on Erewhon Station, is on a steep gully face perpendicular to the main river valley. A fence has been erected around its perimeter to prevent livestock access, though evidence of herbivory is present within. The Lawrence Valley is known to provide excellent ungulate hunting opportunities, and evidence of herbivory is also present within the larger stand. Goats and brush-tailed possums were seen while traversing the site.

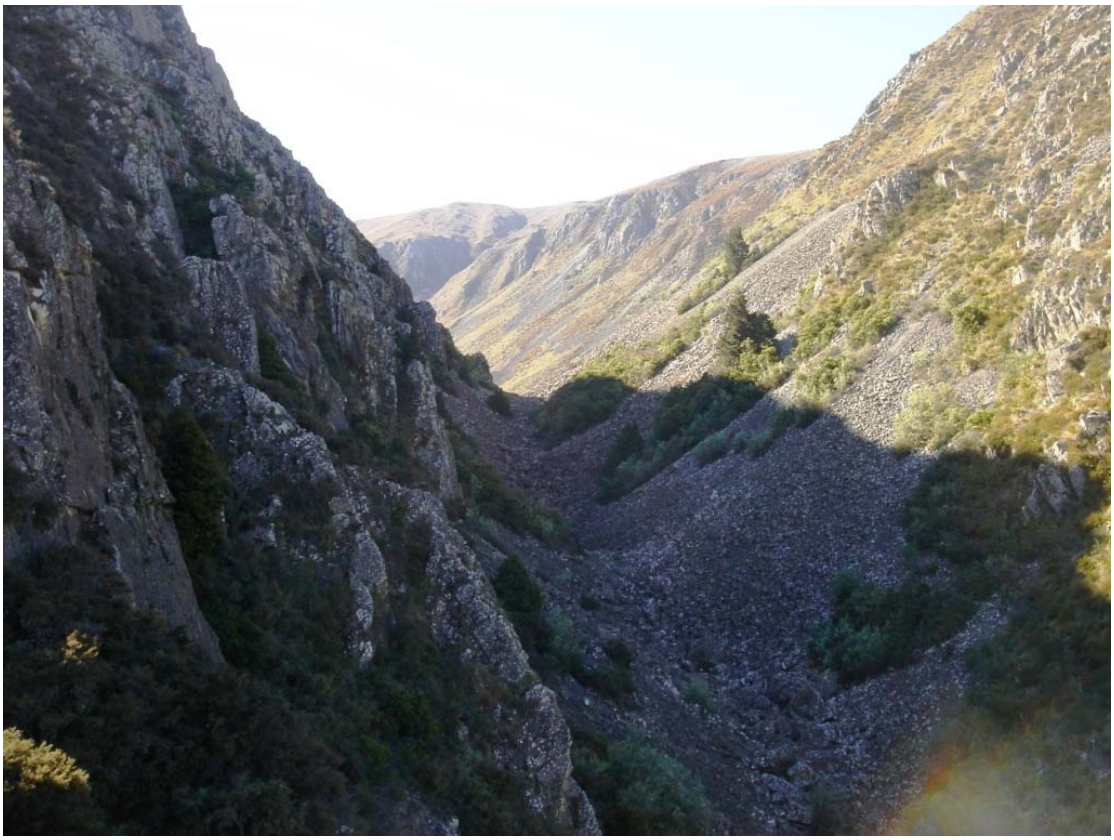

Plate 3.4 – Scattered clumps of *Podocarpus cunninghamii* woodland at Dogs Range.

This site lies at the southern end of the Dogs Range, approximately 3 km north of Lake Clearwater, just north of Mt Guy; it is part of the Hakatere Conservation Area. The area's forest cover has been dramatically reduced to disconnected clumps of trees and shrubs within steep rock fields (Plate 3.4). Common and distinctive trees and shrubs include *Podocarpus cunninghamii*, *Phyllocladus alpinus, Hoheria lyallii, Coprosma linariifolia, Coprosma propinqua, Corokia cotoneaster* and *Myrsine divaricata*. Old *Podocarpus cunninghamii* logs can be found scattered about the rock fields and grassy areas; these lay testament to a bygone period of more extensive woodland cover. A few very large individual trees (dbh > 1 m) are suggestive that the majority of the current population are the result of regeneration since a previous disturbance event. If this site remains free of severe disturbance it seems likely that the gaps currently present between the clumps of woody vegetation will eventually be in-filled by *Podocarpus cunninghamii* forest. The site is subject to mammalian herbivory, with a group of Himalayan thar seen.

3.3.1.5 Mt Dobson (MD)

The Mt Dobson site, situated just under 20 km northwest of Fairlie and approximately 10 km east of Lake Tekapo, lies within Te Kahui Kaupeka Conservation Park, formerly known as the Mt Dobson Conservation Area, at the southern end of the Two Thumb Range.

The stand is present on a very steep gully face (approx. 45°) with rock outcrops at the top of the site. The canopy is dominated by *Podocarpus cunninghamii, Griselinia littoralis* and *Pseudopanax crassifolius*, with *Podocarpus cunninghamii* also present as emergents (Plate 2.4). Trees and shrubs such as *Pseudopanax colensoi* var. *ternatus, Coprosma linariifolia* and *Coprosma propinqua* all occur frequently. The site lies within subalpine grassland and scrub, and extensive cutting of wilding conifers has taken place on adjacent land. Wallabies and possums were seen, with extensive wallaby tracks being common throughout, particularly towards the top of the site.

3.3.1.6 Aoraki/Mt Cook National Park (MC)

Two distinct stands of *Podocarpus cunninghamii* dominated communities were sampled. The larger of the two is sited on the steep (approx. 45°), east facing mountainsides of the Mt Cook Range, at the end of the Tasman Valley Road overlooking Tasman Lake. The second, smaller site is located directly northwest of Mt Cook Village, on a steep face (approx. 35°) of the Sealy Range.

The first site is covered in dense forest, grading into thick subalpine scrub at higher altitudes. *Podocarpus cunninghamii* is common within the canopy and as an emergent (Plate 3.5). The canopy itself is dominated by *Griselinia littoralis, Pseudopanax colensoi* var. *ternatus* and *Phyllocladus alpinus*, with *Dracophyllum longifolium* increasing in abundance higher up the slope. Large specimens of *Podocarpus cunninghamii*, though present, are far from common, with trees most often being 5-8 m tall.

The second site covers a much smaller area and is far less dense; however it does support larger specimens of *Podocarpus cunninghamii* and suggests that the site has either been clothed in *Podocarpus cunninghamii* forest for a longer time than the first,

or perhaps more likely, that the site is more stable, with less frequent rock fall or slippage (Plate 2.5). The bottom edge of this site is highly invaded by exotic herbs, with *Mycelis muralis* and *Digitalis purpurea* being particularly common.

Plate 3.5 – Dense forest with emergent *Podocarpus cunninghamii*, overlooking Tasman Lake.

3.3.1.7 Godley Peaks (GP)

This site is situated on Godley Peaks Station, along the south eastern faces of the Halls Range, overlooking Lake Tekapo. The site is relatively steep, with numerous sloping fans often reaching over 30°. The distribution of vegetation is very patchy, with the patches also being small (Plate 3.6).

Where *Podocarpus cunninghamii* is present within vegetation patches it tends to form an emergent canopy layer over trees and shrubs such as *Hoheria lyallii, Corokia cotoneaster, Coprosma propinqua*, *Aristotelia fruticosa* and *Pittosporum anomalum*. Small individual specimens of *Podocarpus cunninghamii* are present emerging out of dense shrubland areas. The canopy at this site is considered to be that created by

Podocarpus cunninghamii, with the shrubs constituting an understorey layer. Typical canopy species such as *Griselinia littoralis* or *Pseudopanax crassifolius* are absent. Herbaceous and grass species more typically associated with open areas, such as *Festuca novae-zealandiae*, are common. There is evidence of past logging, with several old cut stumps present.

Plate 3.6 – Patch of *Podocarpus cunninghamii* woodland within a stable rock field, at Godley Peaks.

3.3.1.8 Mt Ben Ohau (MB)

This site is characterised by numerous rock fields, broken only by patches of grassland, shrubland or *Podocarpus cunninghamii* woodland (Plates 2.6 and 3.7). Despite the patchy nature of the vegetation, this site represents the largest population of *Podocarpus cunninghamii* in the Mackenzie Ecological Region (Espie *et al.*, 1984). Where enough trees are present to form a canopy, *Podocarpus cunninghamii* is the only canopy tree. Typical canopy forming species, such as *Griselinia littoralis* and *Sophora microphylla*, are only represented here by a few individuals; *Phyllocladus alpinus* is conspicuous by its absence. The shrub component is diverse and often

extremely dense. Common species include *Discaria toumatou, Coprosma propinqua, Corokia cotoneaster* and *Aristotelia fruticosa*. As with Godley Peaks, a more diverse community of grassland plants is present, including native species, such as *Aciphylla aurea*, and invasive exotic species, such as *Hieracium pilosella*.

Plate 3.7 – Patchwork of *Podocarpus cunninghamii* woodland, dense shrubland, grassland and stable rock fields, at Mt Ben Ohau.

3.3.1.9 Birdwood Station (BS)

Part of the Diadem Range, this site lies on Birdwood Station, near Omarama. Four stands of *Podocarpus cunninghamii* dominated communities occur side by side, within shallow, steep (approx. 35°) and rocky gullies on the hill face overlooking State Highway 8.

Where *Podocarpus cunninghamii* forms a canopy it is dense, and other vascular plant species are rare; however the shrub community can be quite diverse within areas of thin canopy or light gaps. Species such as *Coprosma propinqua, Corokia cotoneaster*, *Aristotelia fruticosa* and the invasive *Rosa rubiginosa* are common;

Phyllocladus alpinus is again conspicuous by its absence. Scattered shrubs, often *Discaria toumatou*, are present in the pasture surrounding the stands and appear to have a positive association with the appearance of *Podocarpus cunninghamii* seedlings. Natural regeneration of *Podocarpus cunninghamii* and other native shrubs is evidence of the gaps between the stands slowly beginning to in-fill (Plate 2.7). This is in spite of ongoing sheep grazing on these faces, with no fencing having been erected to prevent stock access to the woodland areas.

3.3.1.10 Longslip Station (LS)

Three small populations of *Podocarpus cunninghamii* dominated vegetation exist on Longslip Station, all overlooking State Highway 8, just 3 km west of the Birdwood Station populations. As with Birdwood Station, each stand occurs within adjacent shallow, steep (approx. 30°) and rocky gullies, surrounded by pasture (Plate 3.8). Shrubs are common only within *Podocarpus cunninghamii* light gaps, including *Ribes uva-crispa*, which abounds. *Phyllocladus alpinus* is here conspicuous by its presence. Sheep grazing also takes place here.

Plate 3.8 – Remnant *Podocarpus cunninghamii* stand at Longslip Station.

3.3.1.11 Lindis (LI)

This site forms part of the Ahuriri Conservation Area and lies adjacent to Longslip Station; access is provided through the Lindis Conservation Area. The site is predominantly steeply sloping (approx. 30°) rock fields with clumps of vegetation scattered throughout (Plate 3.9). *Podocarpus cunninghamii* dominates these vegetation patches with shrubs such as *Coprosma propinqua, Corokia cotoneaster* and *Aristotelia fruticosa* common. Due to the open nature of the site, a diverse herbaceous and grass community is present, with *Hieracium praealtum, Hieracium pilosella* and *Festuca novae-zealandiae* common. Towards the top of the site a small population of *Nothofagus solandri* var. *cliffortioides* is present.

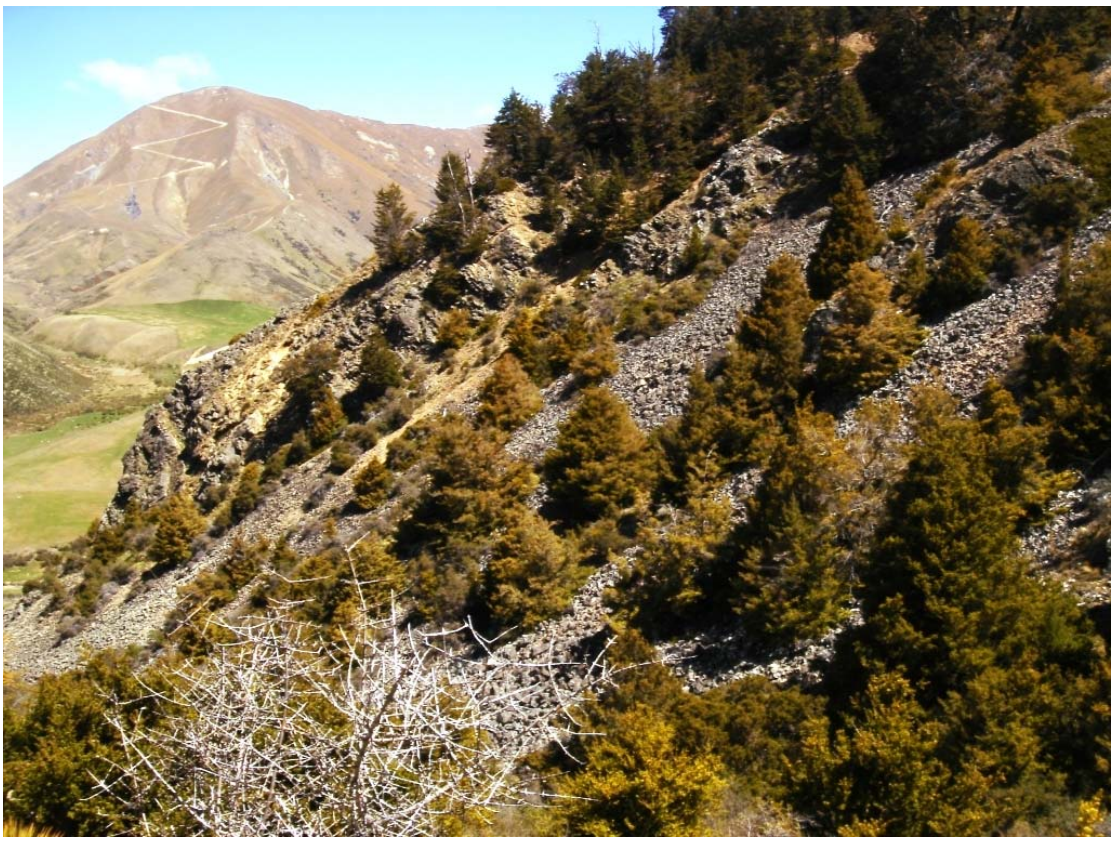

Plate 3.9 – Scattered *Podocarpus cunninghamii* dominated vegetation on a stable rock field at Lindis, with *Nothofagus solandri* trees above.

3.3.1.12 Pisa Range (PR)

The southernmost site studied, Pisa Range is comprised of two distinct populations, one in Alfern Creek Conservation Area and the other in Lochar Burn Scenic Reserve. Both sites are relatively steep (approx. 25°) and differ to the other dryland sites in their lack of rock fields.

Both populations appear seral in nature, with *Kunzea ericoides* forming the vast majority of the habitat and *Podocarpus cunninghamii* apparently slowly increasing in abundance in both the understorey and canopy layers (Plates 2.8 and 3.10). The shrub component is quite species poor, with few species present; typical species include *Coprosma propinqua* and *Gaultheria antipoda*. Exotic shade intolerant grasses such as *Anthoxantham odoratum* are common.

Plate 3.10 –*Kunzea ericoides* scrubland with *Podocarpus cunninghamii*, at Pisa Range.

3.3.2 Vegetation surveys

3.3.2.1 Classification

Seven classes were identified from TWINSPAN analysis. The first division of the analysis separated the dry sites (high annual soil moisture deficit) from the wetter sites (little to no annual soil moisture deficit). For the wetter sites, Mt Cook National Park formed its own class, as did Upper Hapuku; Mt Dobson and Upper Lawrence formed the basis for the third class. For the dry sites, Tone River and Longslip formed the basis for class four; Dogs Range, Godley Peaks and Mt Ben Ohau were all grouped together to form class five; Birdwood Station and Lindis formed the basis of class six; Pisa Range formed class seven (Figure 3.2, Table 3.4). In terms of species diversity (Table 3.4), classes one, two and three have significantly higher Shannon diversity indices than classes four, six and seven, while class five appears intermediate in the diversity split. Classes one, two and three have very few exotic species present, averaging one per plot, while at the other end, classes four and six have significantly more exotic species, averaging between five and six per plot (Table 3.4). The soil properties, environmental attributes and physiognomic characteristics of these classes are shown in Tables 3.5, 3.6 and 3.7, respectively. Classes one, two and three are distinct in having minimal to no annual soil moisture deficit, and similar to the diversity split, class five appears intermediate between the wetter sites and the remaining dry classes with high soil moisture deficit. In terms of soil properties, classes one, two and three have significantly lower levels of P compared with classes four to seven. Other clear differences between the classes are physiognomic, with classes one to three having significantly higher litter and canopy cover values, and classes four to seven having significantly higher rock cover values. A description of each class follows. For a full list of the species present within each vegetation class, together with their percentage cover values see Appendix 1.

Table 3.4 – Site-plot contributions to the different classes, and mean values of species diversity, species richness and exotic species ± 1 s.e. of mean. Different letters within columns indicate significant differences.

Class	Site-plot contributions	Name of vegetation class	Shannon diversity index	Number of species per plot	Number of exotic species per plot
1	UH (1/12) MC (12/15) UL (1/17)	*Podocarpus cunninghamii – Phyllcladus alpinus* forest	2.29 ± 0.08^{a}	17.5 ± 1.6^{ac}	0.6 ± 0.3^{a}
2	UH (11/12)	*Podocarpus cunninghamii –* (*Coprosma linariifolia*) forest	2.25 ± 0.06^{a}	21.4 ± 0.7^{b}	1.3 ± 0.2^{a}
3	MD (6/6) MC (3/15) UL (16/17) DR (1/8)	*Podocarpus cunninghamii –* (*Griselinia littoralis*) forest	2.28 ± 0.06^{a}	18.2 ± 0.8^{a}	0.9 ± 0.2^{a}
4	LI (1/8) LS (5/9) TR (9/9)	*Podocarpus cunninghamii –* (*Phyllocladus alpinus*) woodland	1.95 ± 0.20^{b}	18.3 ± 2.0^{ab}	5.7 ± 0.7^{b}
5	BS (1/12) DR (7/8) GP (7/7) MB (12/15)	*Podocarpus cunninghamii –* (*Corokia cotoneaster*) scrubland	2.13 ± 0.06^{ab}	14.4 ± 0.9^{c}	2.8 ± 0.3^{c}
6	BS (14/15) LI (7/8) LS (4/9) MB (3/15) PR (1/9)	*Podocarpus cunninghamii –* (*Coprosma propinqua*) treeland	1.90 ± 0.08^{b}	16.2 ± 0.7^{ac}	5.6 ± 0.3^{b}
7	PR (8/9)	*Kunzea ericoides – Podocarpus cunninghamii* scrubland	1.92 ± 0.17^{b}	13.5 ± 2.0^{c}	3.9 ± 0.9^{bc}

1) *Podocarpus cunninghamii – Phyllocladus alpinus* forest

This forest type occurs on steep mountain slopes (42º) near the Main Divide, in areas with no annual soil moisture deficit and low minimum winter temperatures (-2.1ºC). It forms a low (approx. 3 m) closed canopy (90% cover), mainly of *Pseudopanax colensoi* var. *ternatus* (24%[2]), *Phyllocladus alpinus* (24%[2]), *Podocarpus cunninghamii* (19%[2]) and *Griselinia littoralis* (14%[2]), and above which *Podocarpus cunninghamii* also emerges (16%[1]). *Dracophyllum longifolium* is also a common component (7%[2]). The shrub layer is sparse beneath the canopy, *Coprosma dumosa* being most common (7%[2]). *Coprosma propinqua* (6%[2]), *Gaultheria crassa* (2%[2]) and *Hebe subalpina* (2%[2]) are common in canopy gaps. The ground flora is mainly comprised of regenerating canopy species, such as *Phyllocladus alpinus* (13%[3]), *Podocarpus cunninghamii* (12%[3]) and *Grislenia littoralis* (10%[3]). *Polystichum vestitum* is the most common fern (6%[3]) and can form dense areas; *Blechnum penne-marina* (3%[3]) and *Asplenium richardii* (3%[3]) are also common but have a less aggregated presence. Lianes are uncommon, the most common being *Rubus schmidelioides* (0.2%[2]). Although far from common, species more typical of the West Coast are also present, such as *Brachyglottis buchananii* (4%[2]) and *Coprosma serrulata* (0.2%[2]), indicating the high levels of precipitation this forest type receives. The litter layer is well developed (86.4%) and there is a low covering of rock (20%). The soils are highly acidic (4.7 pH) and have relatively low levels of P (8.9 $mg \cdot L^{-1}$). Vascular plant species diversity is relatively high (2.3 Shannon diversity index) with an average of 18 species per plot.

2) *Podocarpus cunninghamii* – (*Coprosma linariifolia*) forest

This forest type occurs in the steep (34º) high precipitation Seaward Kaikoura Range. It does not experience annual soil moisture deficit and has a relatively mild winter minimum temperature (-1.5ºC). The canopy is closed (90%) and formed predominantly by tall (10.5 m) *Podocarpus cunninghamii* trees (70%[1]). The understorey is sparse and populated by *Coprosma linariifolia* (13%[2]), *Phyllocladus alpinus* (8%[2]), *Griselinia littoralis* (7%[2]) and *Carpodetus serratus* (6%[2]); *Pseudopanax crassifolius* (5%[2]) and *Pseudopanax colensoi* var. *ternatus* (2%[2]) have

[1] >3 m height
[2] 0.5 – 3 m height
[3] <0.5 m height

an occasional presence. The most common shrub species is *Hebe traversii* (13%[2]), which is the indicator species for this forest type. Other species common in the shrub layer include *Coprosma propinqua* (11%[2]) and *Coprosma dumosa* (3%[2]). *Astelia petriei* (6%[3]) has a conspicuous presence in the herb layer, as too does the exotic *Mycelis muralis* (7%[3]). However, the herb layer is dominated by the fern *Polystichum vestitum*, which can form dense and extensive mats (68%[3]); *Blechnum montanum* is also common (15%[3]). The most common liane is *Rubus cissoides* (6%[2]); although *Clematis forsteri* is also present (4%[2]). The litter layer is well developed (88%) and there is a high covering of ground flora (85%). The soil is acidic (5.8 pH) with an intermediate level of available P (15.0 $mg \cdot L^{-1}$). Vascular plant species diversity is relatively high (2.3) with an average of 21 species per plot.

3) *Podocarpus cunninghamii* – (*Griselinia littoralis*) forest

This forest type occurs close to the Main Divide on steep valley faces (35º) with minimal water deficit and low winter minimum temperature (-2.1ºC). The closed canopy (91%) stands approximately 5 m height and is formed mainly by angiosperm species; *Griselinia littoralis* (16%[1]), *Coprosma linariifolia* (10%[2]), *Phyllocladus alpinus* (8%[2]), and *Pseudopanax crassifolius* (5%[2]) are the most common. *Podocarpus cunninghamii* is also present in the canopy and, more frequently, emerges above it (33%[1]). The understorey and shrub layer is diverse, especially in canopy gaps, containing such species as *Coprosma propinqua* (5%[2]), *Hoheria lyallii* (3%[2]), *Pittosporum tenuifolium* (3%[2]) and *Myrsine divaricata* (2%[2]). The herb layer is rich in ferns, including *Polystichum vestitum* (23%[3]), *Microsorum pustulatum* (9%[3]), *Blechnum montanum* (5%[3]), *Blechnum penne-marina* (5%[3]). *Pyrrosia eleagnifolia*, more typical of the West Coast, is also present (1%[3]). Other species common in the herb layer are *Lagenifera strangulata* (3%[3]) and *Uncinia clavata* (3%[3]), as well as regenerating woody species, such as *Griselinia littoralis* (8%[3]), *Coprosma linariifolia* (5%[3]) and *Podocarpus cunninghamii* (5%[3]). *Rubus cissoides* (1%[2]) and *Rubus schmidelioides* (1%[2]) are the most common lianes. The litter layer is well developed (87%), the soil is acidic (5.3 pH) with low-intermediate levels of available P (12.7

[1] >3 m height
[2] 0.5 – 3 m height
[3] <0.5 m height

mg·L^{-1}). Vascular plant species diversity is relatively high (2.3), with an average of 18 species per plot.

Table 3.5 – Soil properties of the different classes ± 1 s.e. of mean. Different letters within columns indicate significant differences.

Class	**pH**	**Soil N** (%)	**Soil C** (%)	**C:N ratio**	**Olsen P** (mg·L^{-1})
1	4.7 ± 0.1^{a}	0.47 ± 0.01^{a}	8.27 ± 0.13^{a}	17.7 ± 0.2^{a}	8.9 ± 0.6^{a}
2	5.8 ± 0.0^{b}	0.45 ± 0.00^{a}	6.90 ± 0.00^{a}	15.3 ± 0.0^{b}	15.0 ± 0.0^{b}
3	5.3 ± 0.1^{c}	0.56 ± 0.02^{b}	8.47 ± 0.21^{a}	15.3 ± 0.3^{b}	12.7 ± 0.6^{b}
4	5.9 ± 0.1^{b}	0.41 ± 0.01^{a}	6.45 ± 0.18^{a}	15.6 ± 0.2^{b}	21.5 ± 1.4^{c}
5	6.3 ± 0.1^{d}	0.79 ± 0.06^{c}	11.90 ± 1.29^{b}	14.3 ± 0.5^{c}	21.1 ± 1.1^{c}
6	5.6 ± 0.1^{e}	0.39 ± 0.01^{a}	5.41 ± 0.11^{c}	14.0 ± 0.2^{c}	21.0 ± 0.9^{c}
7	5.2 ± 0.02^{c}	0.33 ± 0.01^{a}	5.63 ± 0.08^{c}	17.1 ± 0.1^{a}	32.0 ± 0.0^{d}

4) *Podocarpus cunninghamii* – (*Phyllocladus alpinus*) woodland

This woody community occurs on high altitude mountain slopes (950 m) in areas of very high annual soil water deficit (111 mm·yr^{-1}). The mean annual temperature (7.5ºC) is significantly lower than all the other classes apart from class seven, and it experiences very low winter minimum temperatures (-3.2ºC). The canopy, which stands at approximately 6 m and is not continuous (60%), is comprised mainly of *Podocarpus cunninghamii* (38%[1]), but includes *Phyllocladus alpinus* (17%[2]), *Pittosporum tenuifolium* (5%[2]) and *Hoheria lyallii* (2%[2]), which often intergrade into a sub-canopy. In places where the canopy does close it can be dense, with little growing in the shrub layer, however, as the canopy is often open, the shrub layer of the community can be quite diverse. Species such as *Coprosma propinqua* (5%[2]), *Hebe subalpina* (4%[2]) and *Olearia nummulariifolia* (3%[2]) are common; in the Inland Kaikoura Range *Hebe traversii* is the dominant shrub (8%[2]), and *Brachyglottis monroi* (2%[2]) is a local component. The exotic shrubs *Ribes uva-crispa* (5%[2]), which is an indicator species for this community, and *Rosa rubiginosa* (2%[2]) are common. The ground flora consists largely of sprawling *Podocarpus cunninghamii* foliage (20%[3]) and shrub regeneration, such as *Phyllocladus alpinus* (11%[3]) and *Ribes uva-crispa* (9%[3]). The most common herbaceous species are exotic, including *Hieracium*

[1] >3 m height
[2] 0.5 – 3 m height
[3] <0.5 m height

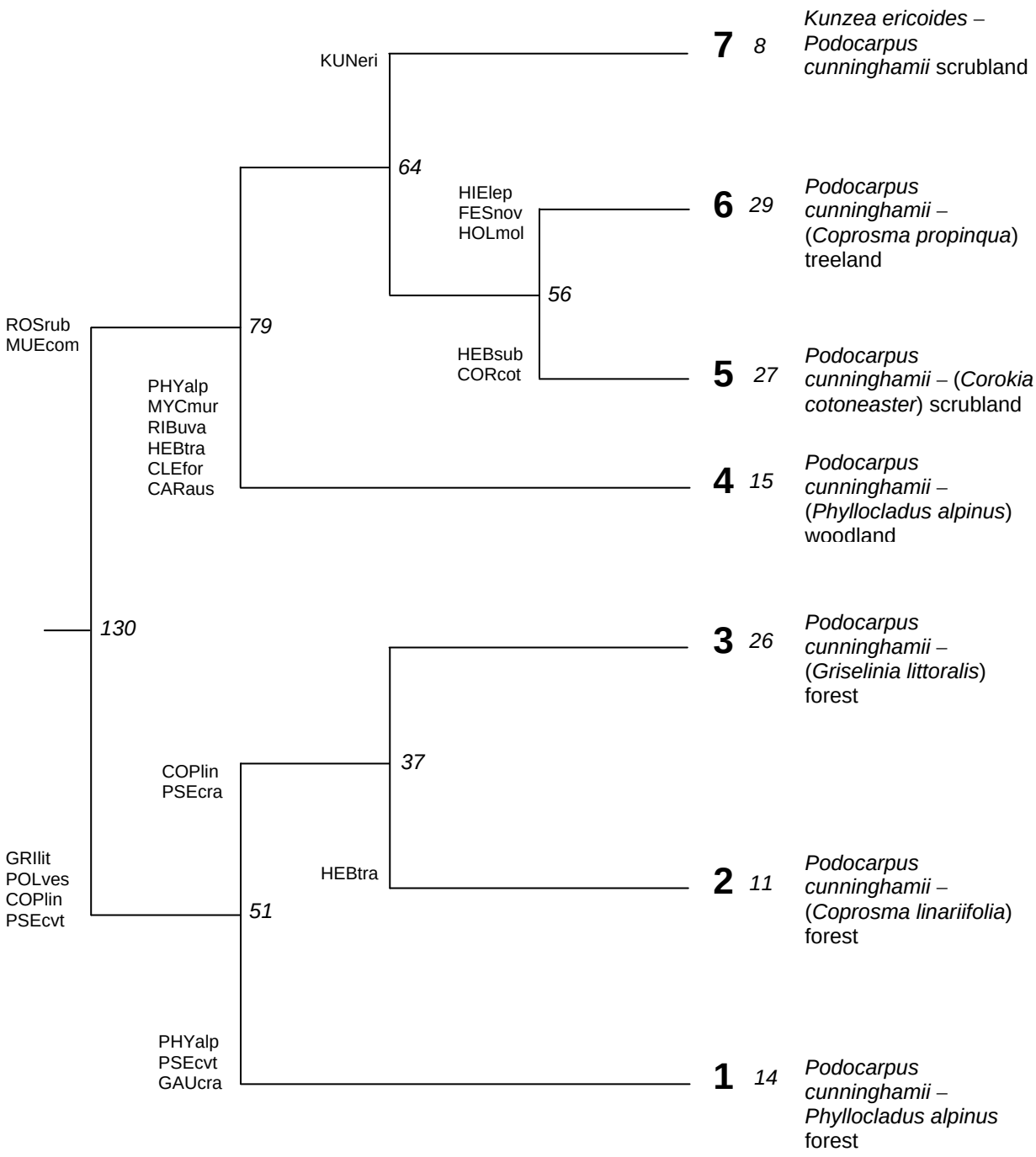

Figure 3.2 – Seven class dendrogram derived from TWINSPAN analysis. Indicator species are listed along the branches of each division, according to NVS six letter codes. The number of plots at each division and within each class is given in italics. The seven classes are listed in bold on the right-hand side.

Table 3.6 – LENZ environmental variables of the different classes ± 1 s.e. of mean. Different letters within columns indicate significant differences.

Class	**Aspect** (°)	**Slope** (°)	**Altitude** (m)	**Water deficit** (mm)	**Vapour pressure deficit** (kPa)	**Mean temp** (°C)	**Mininmum temp** (°C)	**Solar radiation** ($MJ \cdot m^{-2} \cdot d^{-1}$)
1	89.2 ± 5.0^{a}	42.1 ± 1.6^{a}	862 ± 21^{a}	0.0 ± 0.0^{a}	0.40 ± 0.008^{a}	8.0 ± 0.1^{a}	-2.3 ± 0.04^{a}	13.9 ± 0.06^{a}
2	124.3 ± 4.5^{b}	34.4 ± 3.5^{b}	906 ± 22^{b}	0.0 ± 0.0^{a}	0.50 ± 0.005^{b}	8.1 ± 0.1^{a}	-1.5 ± 0.1^{b}	14.6 ± 0.00^{b}
3	90.0 ± 9.3^{a}	34.7 ± 1.6^{b}	836 ± 9^{a}	0.4 ± 0.4^{a}	0.42 ± 0.005^{c}	8.0 ± 0.03^{a}	-2.1 ± 0.04^{c}	13.8 ± 0.05^{a}
4	115.5 ± 6.0^{b}	33.8 ± 1.6^{b}	928 ± 38^{b}	111.4 ± 6.9^{b}	0.44 ± 0.006^{d}	7.5 ± 0.1^{b}	-3.2 ± 0.05^{d}	14.4 ± 0.13^{b}
5	123.2 ± 6.0^{b}	35.7 ± 0.7^{b}	831 ± 14^{a}	42.0 ± 6.4^{c}	0.44 ± 0.005^{d}	8.0 ± 0.1^{a}	-2.3 ± 0.04^{a}	14.0 ± 0.04^{c}
6	131.7 ± 6.5^{b}	32.7 ± 1.1^{b}	750 ± 11^{c}	87.3 ± 3.8^{d}	0.42 ± 0.003^{c}	8.1 ± 0.1^{a}	-3.0 ± 0.05^{e}	13.8 ± 0.01^{a}
7	76.0 ± 13.1^{a}	26.6 ± 2.8^{c}	808 ± 21^{a}	95.5 ± 7.0^{b}	0.40 ± 0.006^{a}	7.6 ± 0.1^{b}	-3.6 ± 0.04^{f}	13.8 ± 0.02^{a}

Table 3.7 – Stand characteristics of the different classes ± 1 s.e. of mean. Different letters within columns indicate significant differences.

Group	**Vegetation** (%)	**Non-vascular** (%)	**Litter** (%)	**Bare ground** (%)	**Rock** (%)	**Canopy** (%)	**Top height** (m)
1	42.5 ± 5.1^{a}	25.4 ± 3.8^{a}	86.4 ± 3.6^{a}	1.4 ± 1.4^{ab}	20.0 ± 5.1^{a}	87.9 ± 3.9^{a}	3.1 ± 0.2^{a}
2	85.5 ± 3.7^{b}	11.4 ± 2.2^{b}	88.2 ± 1.2^{a}	0.5 ± 0.5^{ab}	1.4 ± 3.7^{b}	90.0 ± 3.0^{a}	10.5 ± 1.4^{b}
3	61.0 ± 5.1^{c}	20.6 ± 2.3^{a}	87.1 ± 1.7^{a}	1.7 ± 0.9^{ab}	25.4 ± 5.1^{a}	91.2 ± 2.9^{a}	5.2 ± 0.4^{c}
4	54.0 ± 7.9^{c}	23.0 ± 4.9^{a}	41.0 ± 5.3^{b}	4.7 ± 1.6^{a}	52.0 ± 7.9^{c}	61.3 ± 6.8^{b}	5.7 ± 0.5^{c}
5	42.0 ± 3.4^{a}	19.4 ± 3.6^{a}	49.1 ± 3.6^{b}	0.0 ± 0.0^{b}	89.1 ± 3.4^{d}	46.7 ± 3.5^{b}	4.4 ± 0.2^{c}
6	50.3 ± 3.7^{c}	10.9 ± 1.8^{b}	37.6 ± 3.7^{b}	4.1 ± 1.8^{a}	60.9 ± 3.7^{c}	44.8 ± 4.0^{b}	5.0 ± 0.3^{c}
7	40.0 ± 9.8^{a}	16.3 ± 3.8^{ab}	40.0 ± 12.4^{b}	8.8 ± 6.1^{a}	56.3 ± 9.8^{c}	61.3 ± 8.5^{b}	2.5 ± 0.2^{a}

lepidulum (15%[3]), *Mycelis muralis* (3%[3]) and *Hieracium praealtum* (3%[3]). *Muehlenbeckia complexa* (3%[2]) and *Clematis forsteri* (2%[3]) are the most common lianes. The litter layer of this community is poorly developed (41%) and rock cover is high (52%). The soil is acidic (5.9 pH) and contains significantly more plant available P than the first three communities (21.5 $mg \cdot L^{-1}$). Species diversity is significantly lower than classes one, two and three (2.0), although it maintains an average of 18 species per plot.

5) *Podocarpus cunninghamii* – (*Corokia cotoneaster*) scrubland

This community type exists on steep slopes (36º) in areas experiencing annual soil moisture deficits (42 $mm \cdot yr^{-1}$) intermediate between classes one to three and classes four, six and seven; and cold winter minimum temperatures (-2.3ºC). The community is very open, with scattered *Podocarpus cunninghamii* (32%[2]) trees standing above a small tree-shrubland mix. Common species include *Hoheria lyallii* (4%[2]), *Corokia cotoneaster* (16%[2]), *Coprosma propinqua* (15%[2]), *Hebe subalpina* (11%[2]), *Melicytus alpinus* (8%[3]), *Aristotelia fruticosa* (5%[2]) and *Discaria toumatou* (5%[2]); *Rosa rubiginosa* (4%[2]) is a common exotic. *Sophora microphylla* (2%[2]), *Griselinia littoralis* (2%[2]) and *Pittosporum tenuifolium* (1%[2]) occur very sporadically. *Podocarpus cunninghamii* has a high ground cover (18%[3]), mainly from sprawling foliage rather than regeneration. The herb layer is comprised predominantly of light demanding exotic species; the two most common being *Hieracium pilosella* (2%[3]) and *Hieracium praealtum* (2%[3]). *Muehlenbeckia complexa* (8%[3]) and *Parsonsia capsularis* (2%[2]) are common lianes. This community has a poorly developed litter layer (49%) and very high rock cover (89%) – in many instances there is no litter visible or detectable to approximately 50 cm depth due to the extensive rock cover. The soils are only mildly acidic (6.3%) and the level of plant available P is relatively high (21.1 $mg \cdot L^{-1}$). Species diversity is intermediate between classes one to three and classes four, six and seven (2.1), although the average number of species per plot is low (14).

[1] >3 m height
[2] 0.5 – 3 m height
[3] <0.5 m height

6) *Podocarpus cunninghamii* – (*Coprosma propinqua*) treeland

This community occurs on moderately steep (33º) hill slopes at a lower altitude to the other classes (750 m). Annual soil water deficits are high (87 mm·yr^{-1}) and the minimum winter temperature is cold (-3.0ºC). The canopy, which is made exclusively of *Podocarpus cunninghamii*, is patchy (46%[2]) and low (5 m); however, where it does close it can be dense, shading out the shrub and herb layers and forming a *Podocarpus cunninghamii* monoculture. Outside of the dense canopy areas *Podocarpus cunninghamii* has a scattered presence, allowing shrubs to proliferate. Common species include *Coprosma propinqua* (16%[2]), *Discaria toumatou* (11%[2]), *Podocarpus nivalis* (7%[3]), *Aristotelia fruticosa* (5%[2]) and *Corokia cotoneaster* (5%[2]); the exotic *Rosa rubiginosa* (5%[2]) is also common. The ground layer is comprised mainly of sprawling *Podocarpus cunninghamii* foliage (29%[3]). The most common herbaceous species are light demanding, and include the native grass *Festuca novae-zelandiae* (7%[3]) as well as the exotics *Hieracium pilosella* (9%[3]), *Hieracium praealtum* (4%[3]), *Hieracium lepidulum* (3%[3]) and *Holcus mollis* (3%[3]). *Rubus schmidelioides* (4%[2]), *Muehlenbeckia complexa* (3%[2]) are the most common lianes. The litter layer is sparse (38%) and rock cover is high (61%). The soils are acidic (5.6 pH) with relatively high levels of plant available P (21.0 mg·L^{-1}). Vascular plant species diversity of this community is the lowest of the seven types (1.9) and it has an average of 16 species per plot.

7) *Kunzea ericoides* – *Podocarpus cunninghamii* scrubland

This scrubland community occurs on relatively shallow slopes (27º) in areas of high annual soil moisture deficit (96 mm·yr^{-1}). The mean annual temperature is low (7.6ºC) and the winter minimum is very cold (-3.6ºC). The canopy is short (2.5 m), is relatively contiguous (61%) and is dominated by *Kunzea ericoides* (47%[2]) and *Podocarpus cunninghamii* (26%[2]). The shrub layer is relatively poor, with *Coprosma propinqua* (6%[2]), *Gaultheria antipoda* (4%[2]), *Discaria toumatou* (3%[2]), *Corokia cotoneaster* (3%[2]) and *Melicytus alpinus* (4%[3]) being the most common species; the exotic *Rosa rubiginosa* (1%[2]) is also present. The ground layer is a mix of woody and herbaceous species. *Kunzea ericoides* (36%[3]) and *Podocarpus cunninghamii* (18%[3])

[1] >3 m height
[2] 0.5 – 3 m height
[3] <0.5 m height

are the most common woody species, while light demanding species such as *Hieracium lepidulum* (21%[3]), *Holcus mollis* (4%[3]), *Dactylis glomerata* (2%[3]), *Agrostis capillaris* (2%[3]) and the native *Poa colensoi* (3%[3]) are the most common herbaceous species. *Rubus schmidelioides* is the most common liane (0.4%[2]), though it is far from abundant. The litter layer is sparse (40%) and bare ground (9%) and rock are common (56%). Vascular plant species diversity is low (1.9) and the average number of species per plot is 14.

3.3.2.2 Ordination

In total, 11 of the 13 environmental variables recorded showed significant ($p<0.05$) correlations with PCA axes one and two (Table 3.8). Annual soil water deficit, mean minimum winter temperature, soil pH and soil P accounted for the majority of the variation in the floristic data on PCA axis one. Mean annual temperature, soil C and soil N accounted for most of the floristic variation on PCA axis two. The relationship of sites, plots and species with the environmental variables is shown in the RDA ordination plots (Figure 3.3, 3.4 and 3.5). In total, RDA axes one and two account for 57.9% of the variation in the data.

Table 3.8 – Pearson's product-moment correlation coefficients calculated between each of the environmental variables and PCA axes 1 and 2. * indicates variables retained for RDA.

Environmental variable	**Correlation coefficients**	
	PCA 1	**PCA 2**
Slope (°)	-0.15	0.16
Aspect (°)*	0.25	-0.26
Altitude (m)*	-0.31	-0.11
Water deficit (mm)*	0.69	-0.46
Vapour pressure deficit (kPa)*	-0.06	0.21
Mean temperature (°C)*	-0.03	0.36
Minimum temperature (°C)*	-0.61	0.54
Solar radiation ($MJ \cdot m^{-2} \cdot d^{-1}$)	-0.13	-0.08
pH*	0.53	0.39
Total C (%)*	-0.01	0.28
Total N (%)*	0.06	0.42
C:N ratio*	-0.39	-0.38
Olsen P ($mg \cdot L^{-1}$)*	0.63	-0.23

[1] >3 m height
[2] 0.5 – 3 m height
[3] <0.5 m height

Table 3.9 – Linear regression results of RDA axes 1 and 2 plot scores against each of the environmental variables. * indicates significant relationship.

Environmental variable	RDA 1		RDA 2	
	***t* - value**	***p* - value**	***t* - value**	***p* - value**
Aspect (°)	3.77	<0.001 *	1.31	0.19
Altitude (m)	3.61	<0.001 *	2.02	0.046 *
Water deficit (mm)	11.19	<0.001 *	6.16	<0.001 *
Vapour pressure deficit (kPa)	0.70	0.48	1.12	0.27
Mean temperature (°C)	0.54	0.59	4.75	<0.001 *
Minimum temperature (°C)	9.10	<0.001 *	7.00	<0.001 *
pH	7.17	<0.001 *	4.22	<0.001 *
Total C (%)	0.18	0.86	4.08	<0.001 *
Total N (%)	0.63	0.53	6.25	<0.001 *
C:N ratio	4.77	<0.001 *	4.99	<0.001 *
Olsen P ($mg \cdot L^{-1}$)	9.63	<0.001 *	3.82	<0.001 *

Pearson's product-moment tests of RDA axes one and two with PCA axes one and two shows that they are highly correlated (RDA1 vs PCA1 = -0.999; RDA2 vs PCA2 = 0.915). Linear regressions of the RDA plot scores against the environmental variables shows that RDA axis one has a significant relationship with aspect, altitude, soil water deficit, winter minimum temperature, soil pH, C:N ratio and soil P; RDA axis two has a significant relationship with altitude, soil water deficit, mean annual temperature, winter minimum temperature, soil pH, total C, total N, C:N ratio and soil P (Table 3.9). Annual vapour pressure deficit has no significant relationship with either axis. The RDA ordination biplots (Figures 3.3, 3.4 and 3.5) can therefore be interpreted along axis one as separating sites based more on climatic factors while axis two separates sites based more on soil fertility.

Two main site groupings, separated along axis one, can be discerned from Figure 3.3. Five sub-site groupings can then be identified within the two main groups. The sub-site groupings separate along axis two and show a gradient of floristic turnover within the two main groups. A description of each group follows.

1) Group 1, formed by Mt Cook National Park, Upper Hapuku, Upper Lawrence and Mt Dobson, has very low to no soil moisture deficits. It is characterised by species such as *Griselinia littoralis, Phyllocladus alpinus, Pseudopanax colensoi* var. *ternatus* and *Polystichum vestitum*.

    a. Sub-group 1a is formed predominantly by sites Mt Cook National Park and Upper Hapuku, which are marked by high C:N ratios and by occurring at higher altitudes. They have strongly acidic soils, low winter minimum temperatures and low levels of soil P. Species closely associated with these sites include *Phyllocladus alpinus, Pseudopanax colensoi* var. *ternatus, Coprosma linariifolia* and *Polystichum vestitum*; *Hebe traversii* is common at Upper Hapuku. Sub-group 1a is an agglomeration of classes one and two of the TWINSPAN classification analysis.

    b. Sub-group 1b is formed predominantly by sites Upper Lawrence and Mt Dobson. They are marked by low levels of soil P. Winter minimum temperatures are low, but higher than that of sub-group 1a. Common species are very similar those listed for sub-group 1a, except that *Griselinia littoralis* is the main canopy species. Sub-group 1b is analogous to class three of the classification.

2) Group 2 is formed by Birdwood Station, Dogs Range, Godley Peaks, Lindis, Longslip Station, Mt Ben Ohau, Pisa Range and Tone River. Together they represent the dryland sites. Exotic species are common in this grouping, including *Hieracium* spp. and *Rosa rubiginosa*.

    a. Pisa Range, which forms sub-group 2a, appears as an outlier in the dataset. The canopy is dominated by *Kunzea ericoides* and *Hieracium lepidulum* abounds in the herb layer. This sub-group is marked by having a high level of Olsen P, as well as a low minimum temperature and very high soil moisture deficit. Sub-group 2a is analogous to class seven of the classification.

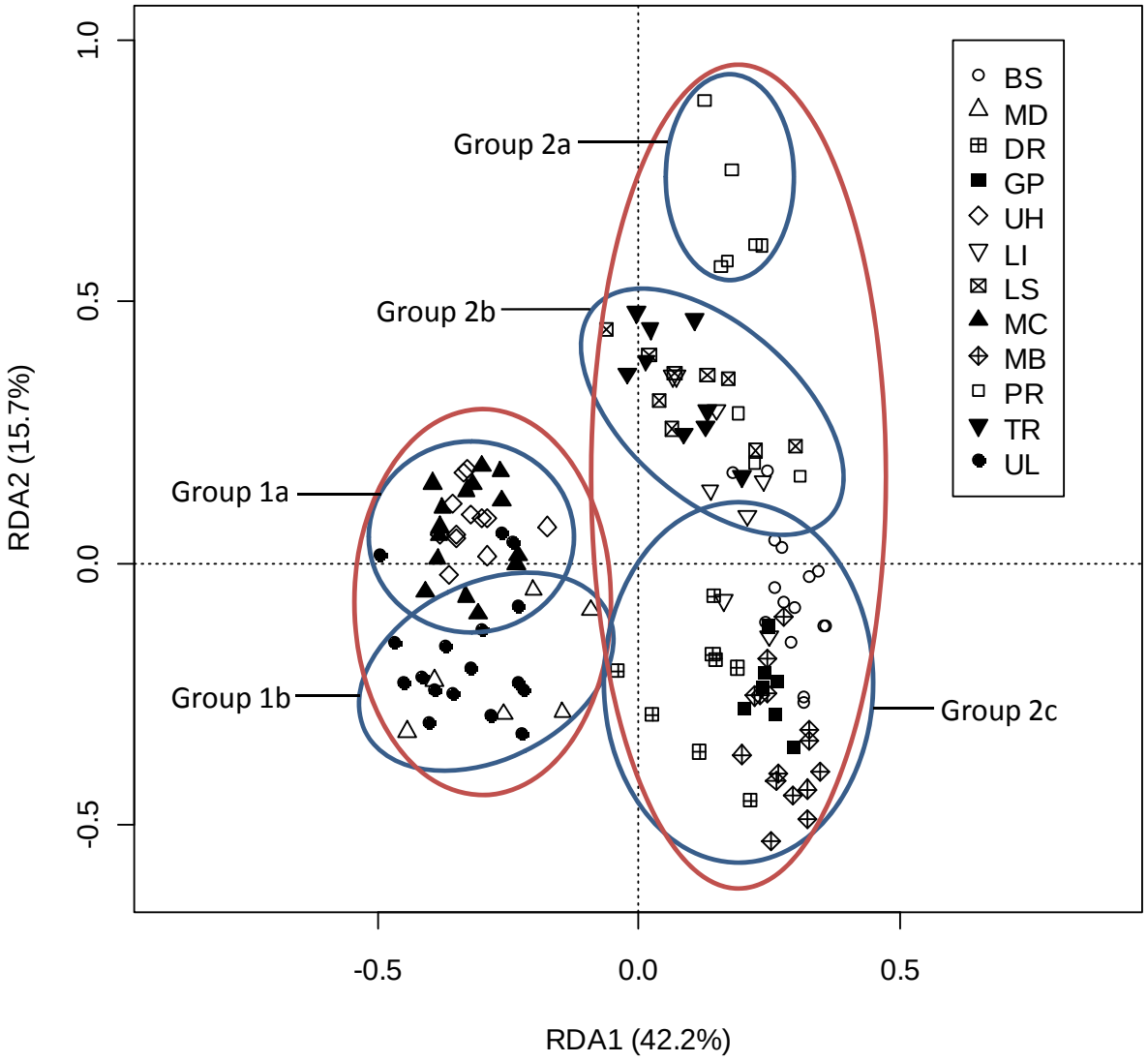

Figure 3.3 – RDA ordination biplot of sites/plots. The main site groups are circled in red. The labelled sub-groups are circled in blue. The total variance explained by each axis is shown in parentheses.

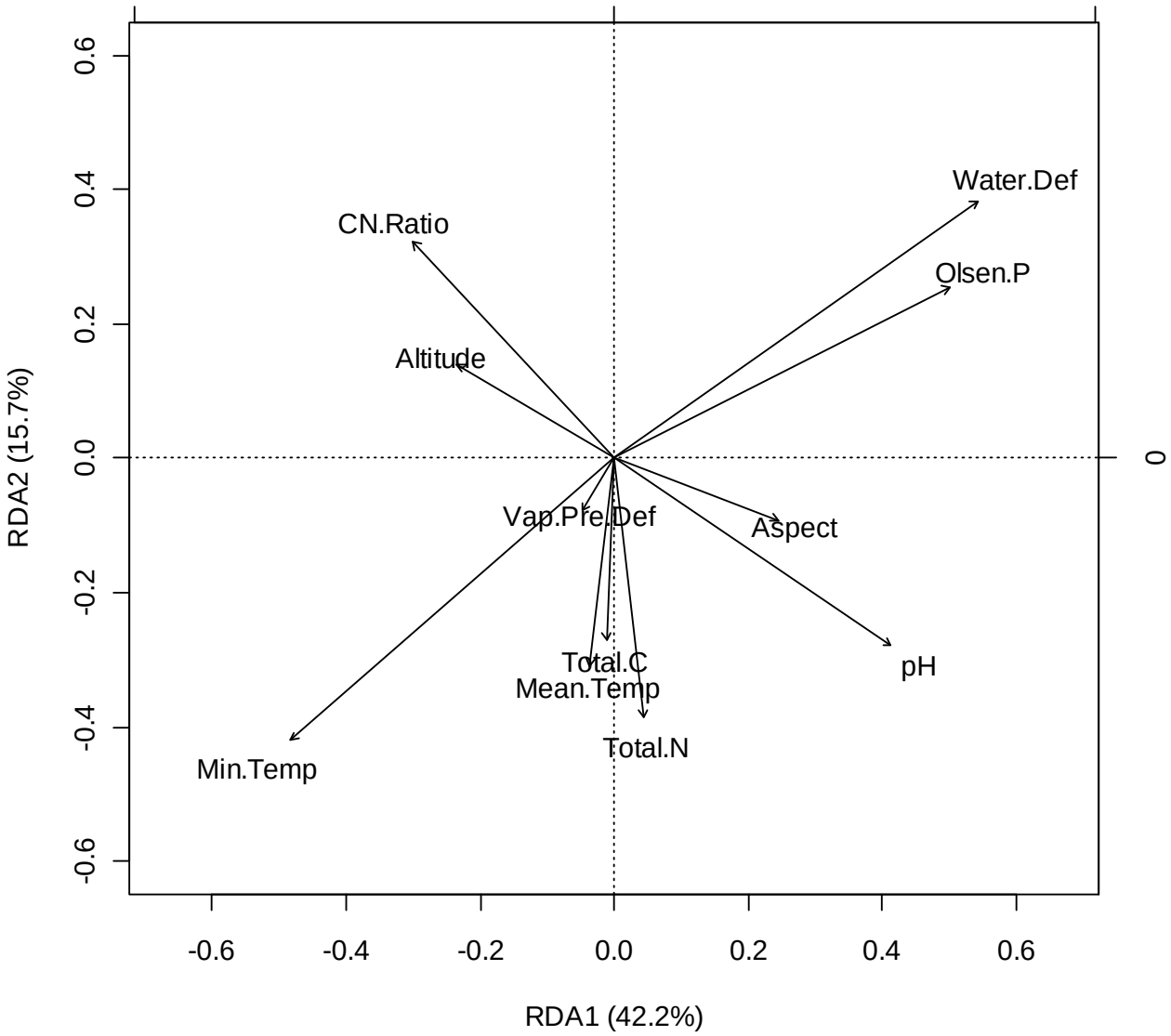

Figure 3.4 – RDA ordination biplot of environmental variables. The total variance explained by each axis is shown in parentheses.

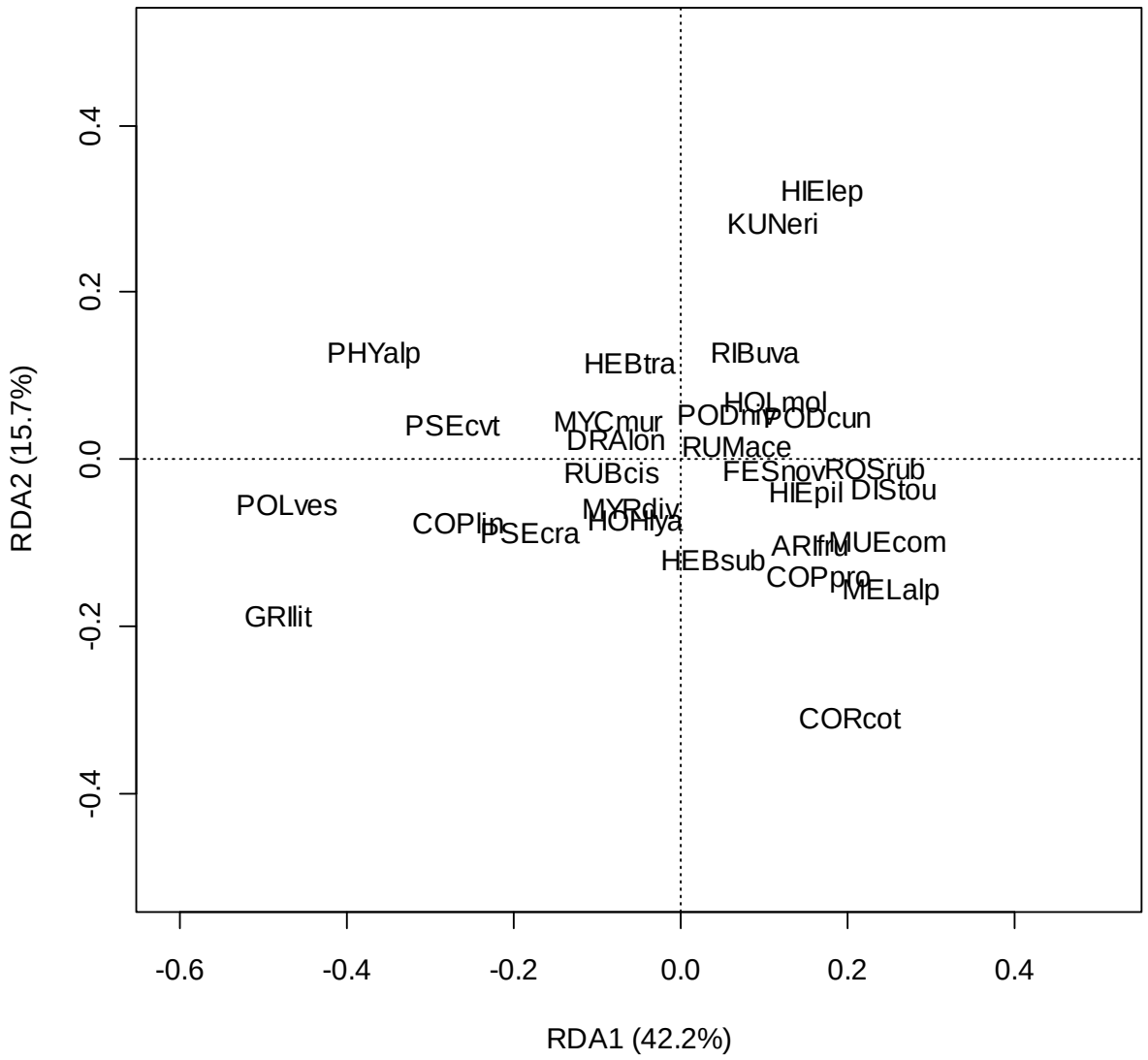

Figure 3.5 – RDA ordination biplot of species. For greater clarity, only the outermost species are shown. Species codes follow the NVS codes (Appendix 2). The total variance explained by each axis is shown in parentheses.

b. Sub-group 2b is made up predominantly by sites Longslip and Tone River. Birdwood Station and Lindis are closely related but intergrade with sub-group 2c. Sub-group 2b has very high soil moisture deficits, high soil P and very low winter minimum temperatures. Closely associated species include *Phyllocladus alpinus, Ribes uva-crispa*, *Pittosporum tenuifolium* and *Hieracium lepidulum*; *Hebe traversii* is common at Tone River. Sub-group 2b is analogous to class four of the classification.

c. Dogs Range, Godley Peaks and Mt Ben Ohau together form the basis of sub-group 2c. Birdwood Station and Lindis are closely related but intergrade with sub-group 2b; the TWINSPAN classification separates Birdwood Station and Lindis as forming their own class (class six). Compared with the sub-groups described above, they have intermediate annual soil moisture deficits and mean minimum temperatures. Shrubland species find close association with these sites, especially *Corokia cotoneaster, Melicytus alpinus* and *Hebe subalpina*. Exotic species are also common, including light-demanding species such as *Rosa rubiginosa* and *Hieracium pilosella*. Sub-group 2c is analogous to class five.

3.3.3 Population dynamics

3.3.3.1 Size-age relationship

Percent age underestimates and the error associated with tree age estimates using dbh were calculated from the three sites where three cores were extracted from each of ten trees. The percentage underestimates of age associated with individual tree cores is shown in Table 3.10; it should be noted that although the standard error decreases dramatically in the largest size class, it is based on just two samples. The age errors associated with dbh are shown in Figure 3.6; error increases with dbh.

Table 3.10 – Percent age underestimates associated with individual tree cores; calculated using three cores from each of ten trees from three sites.

DBH class (cm)	**Mean underestimate** (%)	**Standard error** (%)
5-10 (n = 11 trees)	26.32	3.19
10-20 (n = 11)	20.39	3.43
20-30 (n = 6)	22.84	6.52
30-50 (n = 2)	13.24	0.40

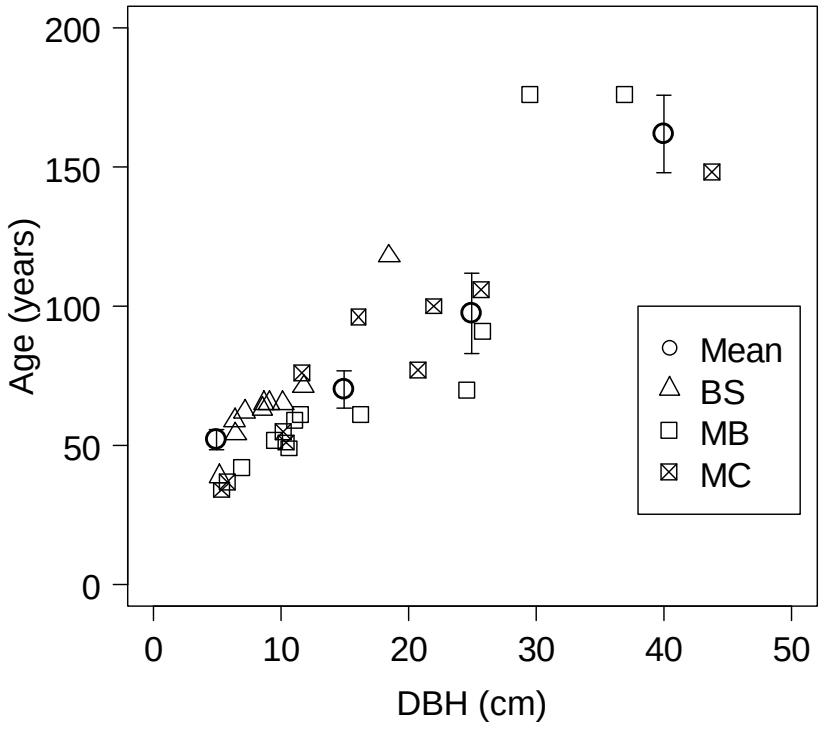

Figure 3.6 – Mean age and errors by dbh. The BS, MB and MC data points show the ages of the oldest of the three cores per tree. The mean data points show the average age of a core within 5-10, 10-20, 20-30 and 30-50 cm dbh classes. Bars show ± 1 s.e.

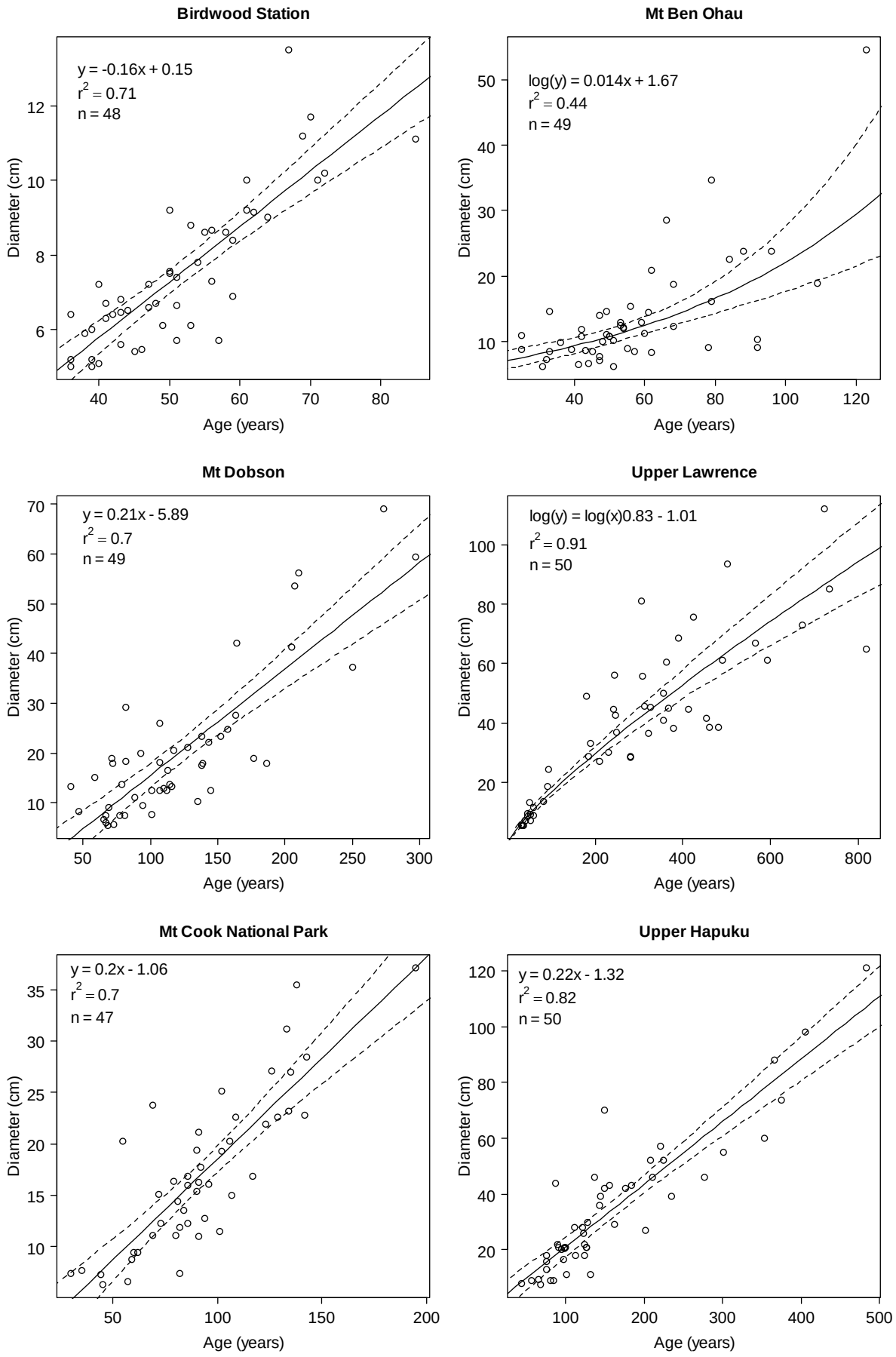

Figure 3.7 – Dbh-age relationships of *Podocarpus cunninghamii* at the six study sites. Dotted lines show 95% confidence intervals.

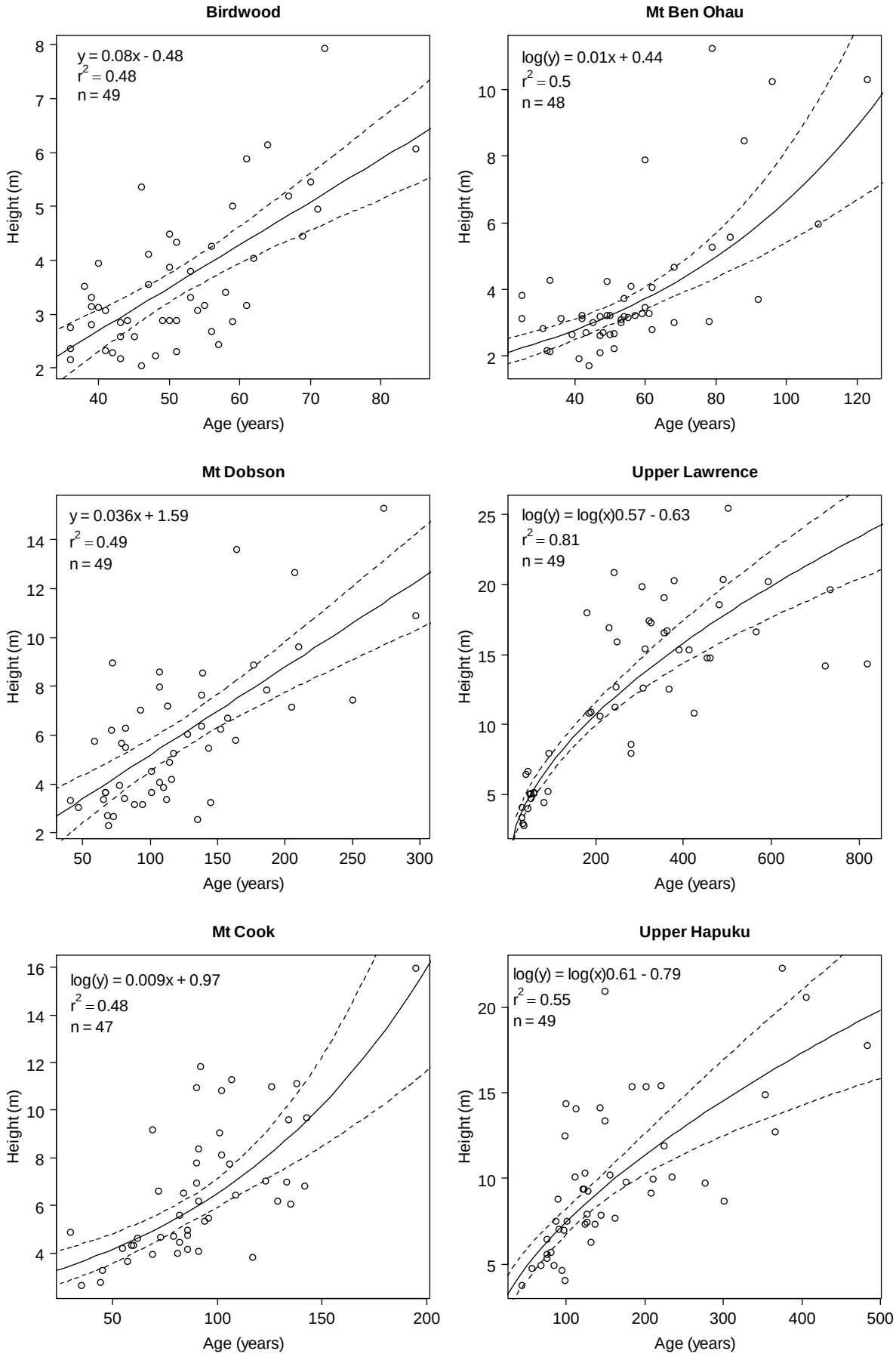

Figure 3.8 – Height-age relationships of *Podocarpus cunninghamii* at the six study sites. Dotted lines show 95% confidence intervals.

3.3.3.2 Size class distributions

The size class distributions of the different sites are shown in Figure 3.9. The Birdwood Station and Mt Ben Ohau sites are populated predominantly with smaller individuals (<20 cm dbh). The populations at Mt Dobson, Upper Lawrence and Mt Cook National Park all have high numbers of saplings present within a mixed sized stand, giving a reverse-J distribution and indicating continuous regeneration to replace canopy trees when such opportunity arises. The population at Upper Hapuku seems to have experienced a distinct episode of regeneration sometime in the past, giving a unimodal distribution peaking in the 20-40 cm size class; there is very little evidence of ongoing recruitment. The size class distribution at Mt Cook National Park is similar to that of Mt Dobson, other than it has a greater abundance of seedlings. The current stem density of this population is very low, and the distribution suggests that *Podocarpus cunninghamii* may be developing a higher stocking rate here.

3.3.3.3 Age class distributions

The age class distributions of the different sites are shown in Figure 3.10. The distributions show marked resemblance to the size class distributions (Figure 3.9), supporting the earlier suggestion that dbh is a reasonable indicator of *Podocarpus cunninghamii* age. The vast majority of trees at sites Birdwood Station and Mt Ben Ohau are less than 100 years old. The distributions of Mt Cook National Park and Mt Dobson are again very similar, with a reverse-J distribution apparent and most individuals being approximately 50-150 years of age. Mt Dobson differs however, as it has numerous individuals with ages well over 200 years. Upper Lawrence shows a bimodal age distribution, with numerous trees present within most age classes, but the majority being either saplings or over 350 years old. Upper Hapuku also provides a bimodal distribution, with the majority of trees within the ages of 50-150 years and the remainder over 350 years; there is very little evidence of regeneration.

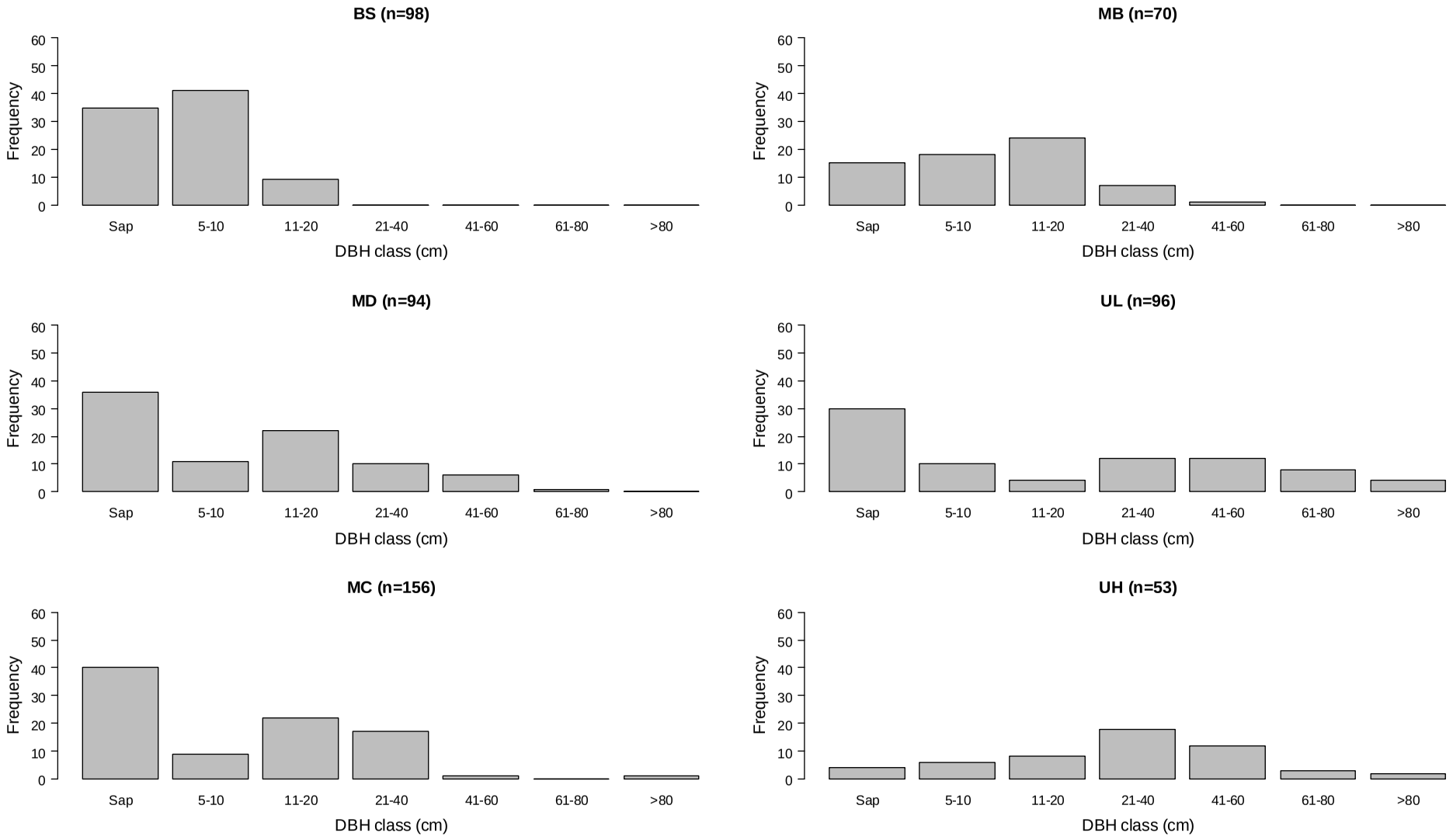

Figure 3.9 – *Podocarpus cunninghamii* size class distributions at the six study sites.

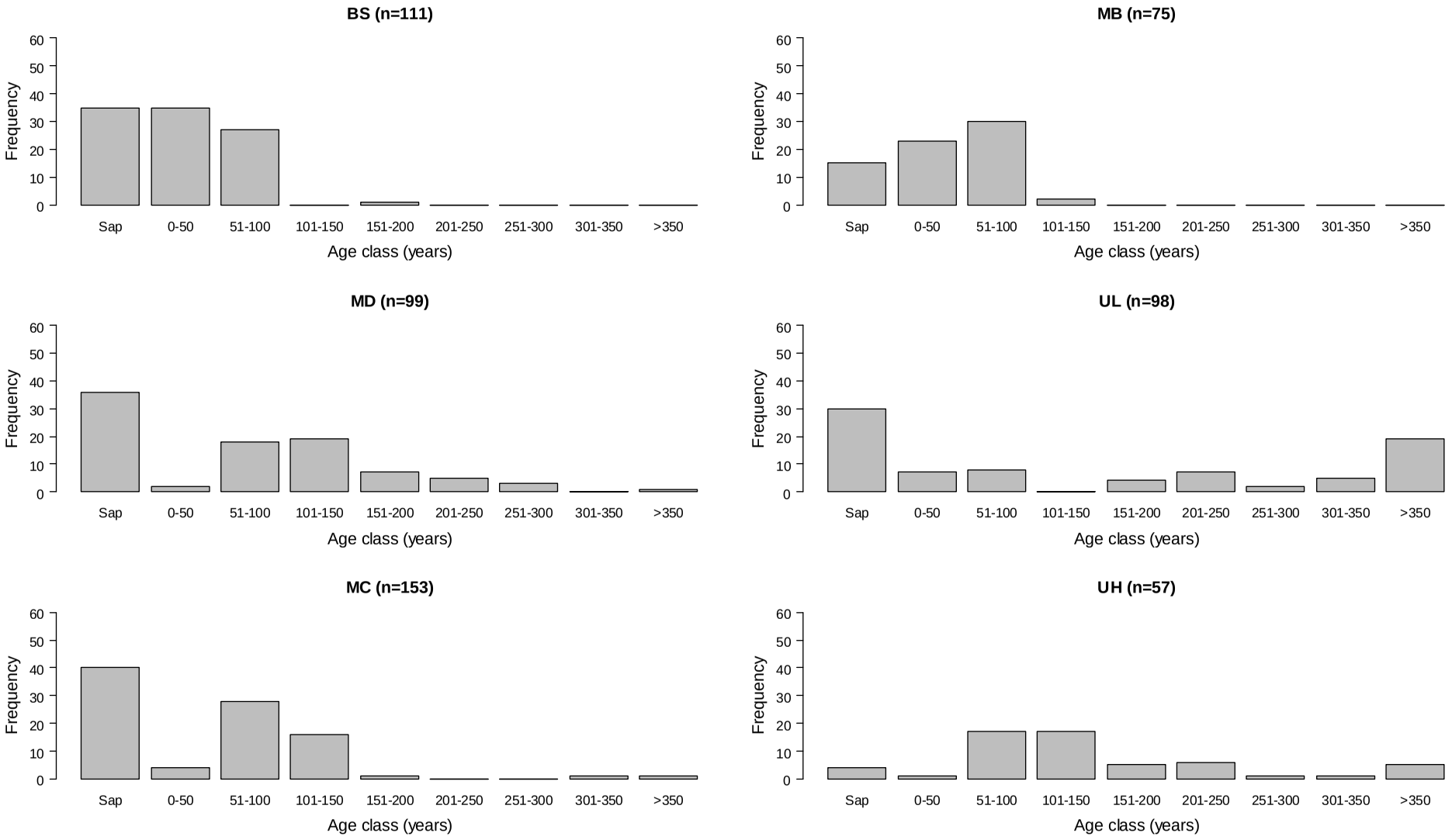

Figure 3.10 – *Podocarpus cunninghamii* age class distributions at the six study sites.

3.3.3.4 Classification

Four classes were identified from UPGMA hierarchical agglomerative cluster analysis of the age class distributions (Figure 3.11). These clustered sites Birdwood Station and Mt Ben Ohau together, Mt Dobson and Mt Cook National Park together, and retained Upper Hapuku and Upper Lawrence as separate classes. These classes highlight the difference in distributions between the sites, with trees at Birdwood Station and Mt Ben Ohau predominantly being in the younger age classes; Mt Dobson and Mt Cook National Park both showing a reverse-J distribution; Upper Lawrence showing continuous regeneration; and Upper Hapuku showing a lack of regeneration.

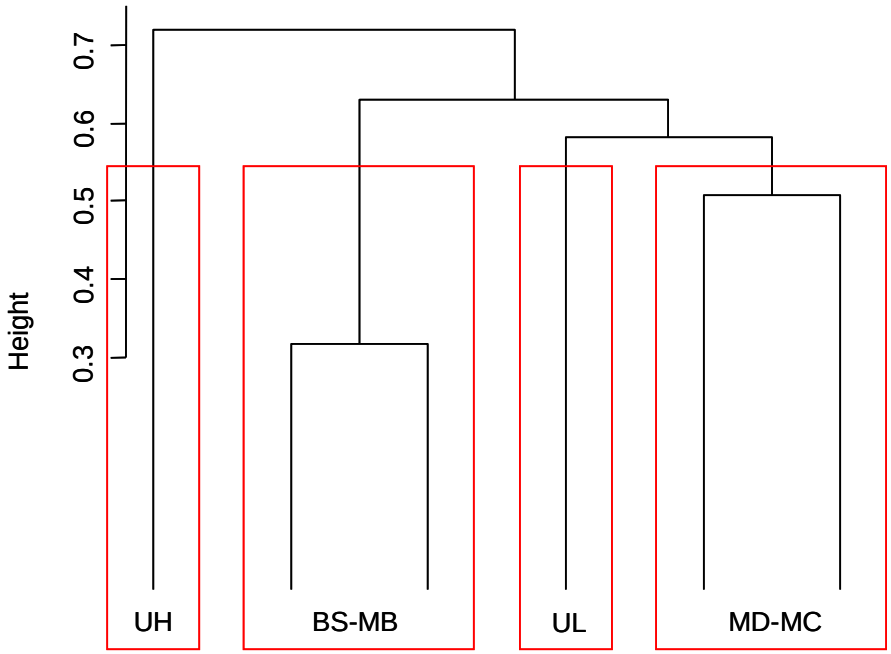

Figure 3.11 – Four class dendrogram, based on *Podocarpus cunninghamii* age class distributions, derived from UPGMA cluster analysis.

3.4 Discussion

3.4.1 Current species distributions and community structure

It is clear from the results that the current distribution of species is strongly affected by environmental gradients. The different sites across the high country show major differences in the species present. Annual soil moisture deficit, mean annual temperatures, aspect, altitude and soil fertility all have a significant influence on these patterns.

Certain species that abound in the wetter parts of the high country either do not occur or have greatly reduced abundances in the drier locations. In wetter regions, forests develop in which *Podocarpus cunninghamii* emerges above canopy forming species such as *Griselinia littoralis, Phyllocladus alpinus, Pseudopanax crassifolius, Pseudopanax colensoi* var. *ternatus* and *Coprosma linariifolia*. The shrub layer of these forests is dominated by shade tolerant species; *Myrsine divaricata, Coprosma dumosa* and *Hebe traversii* are good examples. Many regenerating specimens of species present in the canopy are also present. The herb layer provides habitat for shade tolerant species such as *Polystichum vestitum, Blechnum montanum* and *Lagenifera strangulata*.

In the drier regions, the communities develop a more open woodland or scrubland structure. The patchy nature of the canopy allows increased levels of solar radiation to reach the ground, thereby providing suitable habitat for light demanding species. *Podocarpus cunninghamii* remains the tallest component of the dryland communities and in places is able to form a closed canopy. More commonly however, it develops an intergrading patchwork distribution with numerous tree and shrub species. Species such as *Hoheria lyallii, Coprosma propinqua, Corokia cotoneaster, Aristotelia fruticosa* and *Hebe subalpina* are the most common of the native species, while *Rosa rubiginosa* and, to a lesser extent, *Ribes uva-crispa* are commonly occurring exotics. *Melicytus alpinus* is also common at higher altitudes or with increasing canopy openness. The herb layer too differs markedly to that of the wetter regions; species such as *Hieracium pilosella, Hieracium praealtum, Festuca novae-zelandiae* and *Rumex acetosella*, which are absent from the wet forests, are here plentiful; these species are light demanding, and the open nature of the canopy at these sites no doubt favours their success.

The distribution of certain species, such as *Griselinia littoralis* and *Pseudopanax colensoi* var. *ternatus*, which are typically abundant in the wetter sites and often absent from the drier sites, is not an indication that these species cannot tolerate high soil moisture deficits, or that they were not there historically. Seedlings, saplings and mature individuals of these species can be found at the driest sites. For example, *Griselinia littoralis* is found at Mt Ben Ohau and is also present on the low-rainfall

Hawkdun Range (D.A. Norton, pers. comm.). *Griselinia littoralis* is also known to be able to tolerate high levels of root zone and atmospheric water deficits (Leathwick and Whitehead, 2001). Similarly, *Pseudopanax colensoi* var. *ternatus* is present at Tone River, which is the site recorded as having the greatest annual soil water deficit (Table 3.3). As such, these species are considered to be cosmopolitan and their current disjunct distribution may simply be an artefact of anthropogenic disturbance. Early Polynesian and European fires deforested large swathes of the high country, leaving behind isolated fragments of woody vegetation within a highly modified environment (McGlone, 1989, McGlone, 2001). Those fragments that remain are unlikely to be representative of the pre-human flora due to their small size and highly degraded nature, as well as the fact that they typically exist within specific fire refuges, such as steep gullies and rocky outcrops (Walker *et al.*, 2004b).

The loss of woody cover across the high country due to fire seems the most likely reason for the absence of cosmopolitan species in the dryland remnants. Any species that did manage to survive these fires would have had a greatly reduced abundance. In such cases, mammalian herbivory after fire clearance may have exacerbated the loss of any remaining palatable species. Certainly, it has been said that the native woody species that remain in the drylands are likely the most fire tolerant and unpalatable members of the original flora (Standish *et al.*, 2009). Many of New Zealand's native woody species have evolved little defence to mammalian herbivory due to the absence of terrestrial mammalian herbivores on the archipelago prior to the arrival of humans, and can therefore suffer greatly in terms of growth and reproduction when subject to even moderate levels of grazing (Lee, 1998). Furthermore, *Pseudopanax colensoi* and *Griselinia littoralis* are known to be highly palatable to introduced ungulates (Forsyth *et al.*, 2002, Husheer, 2007, Pollock *et al.*, 2007). All of the dryland sites studied are situated either within active grazing landscapes (cattle and sheep) or on land retired from grazing and now within the public conservation estate. Although the sites within the public conservation estate are no longer subject to livestock grazing, they are still accessible to feral ungulates, including deer, chamois and thar, as well as other herbivores such as hares, rabbits, possums and wallabies (Standish *et al.*, 2009). Therefore, it is possible that these palatable species, assuming they had survived the early fires, could have then been gradually lost over time on sites accessible to livestock and feral herbivores.

The observed differences in the distributions of certain other species, however, are not considered to be an artefact of human disturbance. Woody species such as *Brachyglottis buchananii, Coprosma pseudocuneata, Coprosma rugosa* and *Coprosma serrulata*; herbaceous species such as *Astelia petriei, Lagenifera strangulata, Nematoceras trilobum* and *Pterostylis australis*; and ferns such as *Asplenium flaccidium, Hymenophyllum villosum* and *Pyrrosia eleagnifolia*; all of which were only recorded in plots with minimal soil moisture deficit, are species intolerant of severe water deficits and are therefore confined to sites closer to the Main Divide, or to the coast, in the case of the Seaward Kaikoura Range, where rainfall is higher and soil moisture deficits lower.

In addition to the distribution of indigenous species, the sites show great disparity in their numbers of exotic species. The drier sites all have significantly greater numbers of exotic species compared with the wetter sites, showing that they are more degraded. Although the mean number of species per plot shows no real pattern with soil moisture deficit, the mean number of exotic species per plot does (Table 3.4). When this is taken into account, it is clear that the reason for the similarity in total species numbers between wet and dry sites is due to the high number of exotic species in the drier sites. Species diversity and species richness within the dryland sites would be greatly reduced without the presence of exotic species. Elevated species diversity is often a feature of highly fragmented small communities as a result of species invasions from the surrounding landscape (Cook *et al.*, 2002, Ewers and Didham, 2006). Species such as *Hieracium lepidulum, Hieracium pilosella, Hieracium praealtum, Rumex acetosella, Ribes uva-crispa and Rosa rubiginosa* are all common, and in some cases dominant, amongst the flora of the dryland sites. The interstitial physiognomy of the dryland vegetation, combined with their history of greater anthropogenic disturbance in comparison with the wetter sites, due in part to their dryness, makes them more susceptible to invasion by light demanding exotic species such as *Hieracium* (Rose *et al.*, 1998). The forests found in the wetter sites have greater resistance to invasion by these exotic species as their canopies are more intact, with low levels of solar radiation reaching the ground. The only commonly occurring exotic species within the wetter sites is *Mycelis muralis*, though *Hieracium lepidulum* and *Digitalis purpurea* can be common at forest edges.

3.4.2 Population dynamics

The extent to which individual tree cores underestimate the minimum age of *Podocarpus cunninghamii* trees was higher than expected but reasonably consistent for each age class. In addition, the errors associated with age estimates per dbh size class were relatively low, with age estimates of ± 7 years for those in the 5-10 cm dbh class, increasing to approximately ± 15 years in the 20-30 cm size class (Table 3.10). Given that these errors were pooled from the three different sites, the data suggests relative uniformity in the error rate across the high country. Therefore, despite an increment core providing a 20-25% underestimate of the minimum age of an individual tree, the level of error is consistent across the environments sampled.

In light of this, and in conjunction with the linear regression analyses, diameter at breast height does appear to provide a reasonable approximation of age. However, while on average the standard error of an age estimate may be at most only ± 15 years, it is clear from Figures 3.7 and 3.8 that as dbh increases, age estimates become increasingly inaccurate. This combined with the very low sample sizes of trees in the larger dbh classes means confidence has an inverse relationship with dbh. Height, while having a significant relationship with age, does not provide consistent approximation of tree age and therefore should not be used. It should also be remembered that the age estimates presented here do not take into account the time it takes for *Podocarpus cunninghamii* to reach coring height; thus, the true age of each sampled tree is even greater than the age underestimates calculated.

The age and size class distributions of Birdwood Station and Mt Ben Ohau, which were clustered together in the classification analysis (Figure 3.11), suggest that the stands are relatively young, being, at a minimum underestimate, between 50 and 100 years old. This may be evidence that *Podocarpus cunninghamii* is recolonising these sites after an historic disturbance event. Bearing in mind that all the age estimates underestimates, this disturbance event may have occurred over 100-150 years ago. Furthermore, both these sites have high stem densities (Table 3.2), in particular Birdwood Station (1000 stems·ha^{-1}). This also suggests that these are relatively young stands that will eventually thin to a stem density more or less similar to the other sites (approx. 250 stems·ha^{-1}).

The age and size class distributions of Mt Dobson and Mt Cook National Park are similar to one another (Figure 3.11), apart from Mt Dobson having a lower number of seedlings and a greater number of trees in the older and larger classes. The age-class distributions of both indicate that the bulk of the current stands colonised the site after a disturbance event; as an underestimate, this may have occurred between 50 and 200 years ago. The distributions of Upper Lawrence show a mixed forest of all different age and size classes. It would appear that within forest disturbance occurs every 100-200 years which allows pulses of *Podocarpus cunninghamii* regeneration. This gives the forest numerous small stands that differ in age from one another but are each comprised of similarly aged trees. In addition to this, the presence of large numbers of saplings is similar to Mt Dobson and Mt Cook National Park, in that within the period of larger scale disturbances, which allows recruitment of large numbers of similarly aged *Podocarpus cunninghamii* trees, *Podocarpus cunninghamii* is able to continuously regenerate within the closed canopy of the forest. However, while this regeneration can develop to the sapling stage, in the absence of a small scale disturbance event, e.g. the death of an existing canopy tree that forms a canopy gap, these saplings are unable to attain canopy status. This process is very similar to that described for *Podocarpus cunninghamii* in the North Island (Smale and Kimberley, 1993, Smale and Smale, 2003), and by Veblen & Stewart (1982) elsewhere on the South Island, and suggests that *Podocarpus cunninghamii* stand dynamics are similar across the wide range of environments in which it grows.

The situation at Upper Hapuku contrasts markedly with the other sites. Based on the age underestimates, it appears that large scale recruitment of *Podocarpus cunninghamii* trees occurred sometime over 300 years ago, with several remnant trees still present, and again more recently, between 50 and 250 years ago, peaking at approximately 100-150 years ago. This has resulted in a *Podocarpus cunninghamii* stand that has relatively low size class diversity and is relatively even-aged. There are very few seedlings or sapling present in the understorey, showing that continuous regeneration is not occurring. This might suggest that the forest is developing towards over-maturity, and that *Podocarpus cunninghamii*, being unable to regenerate, will eventually disappear from the emergent layer and be replaced as the canopy dominant by *Griselinia littoralis* and *Pseudopanax crassifolius*, which were

the most common angiosperm species (Holloway, 1954). However, in other parts of the forest, *Podocarpus cunninghamii* did form dense young stands, thereby demonstrating its ability to regenerate within this environment (pers. obs.). The landscape here is very active, as it is tectonically unstable and receives some of the highest rainfall intensities of eastern South Island (Wardle, 1971); the remains of recent landslips, both massive and small, are common. It would therefore appear that *Podocarpus cunninghamii* in the Upper Hapuku has undergone catastrophic regeneration, forming extensive areas of near even-aged stands following massive recruitment after large scale disturbance. Given that *Podocarpus cunninghamii* appears to undergo continuous regeneration at other sites in the high country, the absence or very limited nature of regeneration beneath an established canopy at this site is puzzling, especially when dense clumps of regeneration were observed in other parts of the forest. Light levels, though not quantified at any of the study sites, showed no obvious difference to those of the similar mixed forests at Mt Dobson, Mt Cook National Park or Upper Lawrence. Trampling pressure from introduced ungulates may have reduced seedling numbers, as large numbers are known to be present here (Wardle, 1971), and widespread browsing of angiosperm tree species was common throughout. Possums were also seen here, and may be having a suppressive effect on *Podocarpus cunninghamii* regeneration (Nugent *et al.*, 1997, Nugent *et al.*, 2001). However, despite these possibilities, it seems unlikely that possum browse and ungulate trampling would eradicate *Podocarpus cunninghamii* regeneration over a large forest area, especially as large scale regeneration is taking place. The absence of regeneration beneath mature *Podocarpus cunninghamii* may therefore be a reflection of a different regeneration strategy in this area, given that it is so prone to large scale disturbance.

A diversity of regeneration strategies is common amongst the New Zealand conifers (Ogden and Stewart, 1995). For example, the *Dacrydium cupressinum* forests of the West Coast are known to develop a mosaic structure comprised of groups of even-aged stands, with each stand considered to have arisen from a catastrophic disturbance event (Ogden and Stewart, 1995, and references therein). Within the older stands, patches of even-aged younger trees can often be found, and these arise through gap-phase regeneration when older trees die. It would similarly appear to be the case with *Podocarpus cunninghamii*. Where large scale catastrophic disturbance takes place,

Podocarpus cunninghamii rapidly colonises a site and forms an even-aged canopy or emergent layer. Further *Podocarpus cunninghamii* regeneration can occur through canopy disturbance arising from single or multiple tree fall (gap-phase regeneration) (Smale and Kimberley, 1993, Smale and Smale, 2003), leading to the development of mixed-age stands. In addition, *Podocarpus cunninghamii* is relatively shade tolerant in comparison to New Zealand's other podocarp species (Bergin, 2003, Kunstler *et al.*, 2009), and its seedlings and saplings are able to develop in the understorey, enabling the species to continuously produce potential canopy replacements within a mature forest. As individuals or groups of canopy trees begin to senesce, small scale gap-phase regeneration becomes more important as increased light levels allow these saplings to attain canopy status. In the absence of canopy disturbance, these saplings will remain suppressed or die. Given the relative youth of the sampled dryland stands, it remains unknown whether these same dynamics would take place in mature dryland communities.

3.4.3 Implications for restoration

The stands of *Podocarpus cunninghamii* forests in the wetter regions of the high country appear relatively intact. The stands can be extensive and they have a very low frequency of exotic species. Mammalian herbivores are present within these stands and evidence of herbivory is present in all. However, with the exception of Upper Hapuku, all the stands show evidence of plentiful *Podocarpus cunninghamii* and canopy species regeneration, which suggests that processes fundamental for forest survival are being maintained. As such, these stands provide a clear species template for the ecological restoration of *Podocarpus cunninghamii* forest within the wetter regions of the high country close to the Main Divide. The stand at Upper Hapuku appears subject to more large scale disturbance regimes than Upper Lawrence, Mt Cook National Park and Mt Dobson, and resultantly *Podocarpus cunninghamii* may undergo an alternative regeneration strategy here, thus explaining the lack of its own regeneration beneath mature specimens and its concentration within whole regenerating stands. That being said, evidence of herbivore damage to palatable angiosperm species is plentiful, and may well be inhibiting the regeneration of *Griselinia littoralis, Pseudopanax colensoi* var. *ternatus* and *Pseudopanax crassifolius*, which are present but do not form a contiguous canopy beneath

Podocarpus cunninghamii, as occurs in the other wet sites. Despite this, the difference in *Podocarpus cunninghamii* regeneration dynamics between Upper Hapuku and the other wet forest sites highlights the need for restoration to incorporate and allow disturbance processes within stands to maintain *Podocarpus cunninghamii* population structures.

The stands in the drylands do not offer the same guidance as those in the wetter regions. These stands are small, highly degraded and unlikely to be representative of the pre-human vegetation cover. In general terms, it seems likely that the pre-human dryland landscape, below the alpine zone, would have supported a low forest community, with emergent *Podocarpus cunninghamii* trees between 5-10 m in height, intergrading into a scrubland community with increasing soil moisture deficit. With regard to the low forest community, species such as *Griselinia littoralis*, *Hoheria lyalli*, *Pseudopanax crassifolius*, *Pseudopanax colensoi* var. *ternatus*, *Phyllocladus alpinus*, *Pittosporum tenuifolium* and *Coprosma linariifolia* are likely to have been the main canopy species, though whether the canopy would have been closed is arguably unlikely. Additional species such as *Sophora microphylla* and *Carpodetus serratus* may also have been important (Walker *et al.*, 2004a). Beneath this numerous shrubs would have been present, such as *Myrsine divaricata* and *Coprosma dumosa*. As the forest community thinned to scrubland, with increasing soil moisture deficit, the canopy would have become more open, with species such as *Corokia cotoneaster, Aristotelia fruticosa, Coprosma propinqua, Coprosma rigida* and *Hebe subalpina* becoming more prevalent and the canopy species listed above decreasing in both stature and abundance. Species of *Dracophyllum* and *Gaultheria,* and *Podocarpus nivalis* would have become more important at higher altitudes where the canopy thinned further. Ferns were probably the most abundant form of ground flora, with species of *Polystichum, Asplenium* and *Blechnum* common. For a full species list of each of the classes as well as a guide to individual species abundances, see Appendix 1.

Within the drylands, many of the species listed above are currently either absent or present in extremely low numbers. As such, simply setting land aside for conservation will not lead to its automatic reversion to a *Podocarpus cunninghamii* vegetation community. Active introduction of many species will be necessary. Given

that feral herbivores are present throughout the high country, and the fact that mammalian herbivory can lead to a dramatic decline in palatable New Zealand woody species (Forsyth *et al.*, 2002, Grüner and Norton, 2006, Husheer, 2007, Pollock *et al.*, 2007), thereby causing shifts in community composition and structure (Walker *et al.*, 2009), it is likely that some form of herbivore exclusion will be necessary to ensure the successful restoration of palatable species, e.g. *Griselinia littoralis* and *Pseudopanax*. This is the same for any restoration that takes place within active grazing landscapes, where stock trampling would also limit restoration success.

In light of the regime shifts that have occurred in the drylands since human arrival, it is unlikely that any attempts at restoration will be successful without accepting some component of exotic species also being present (Walker *et al.*, 2009). The woody species *Rosa rubiginosa* and *Ribes uva-crispa*, as well as the herbaceous species *Hieracium lepidulum, Hieracium pilosella, Hieracium praealtum, Mycelis muralis* and *Rumex acetosella* are all likely to be prominent components of any restored community. *Rosa rubiginosa* and *Ribes uva-crispa* are likely to be particularly common and persistent in any restoration attempts, given the scattered and open nature of *Podocarpus cunninghamii* stands in the drylands. The herbaceous species listed are for the most part light-demanding. As such, their presence early on in restoration is likely to be certain; however, if a dense shrub component of the community can be successfully restored it will eventually shade-out these species and thereby reduce their abundance.

The different vegetation classes identified can be used as an indicator of community degradation, and therefore inform on priorities for restoration. Within the drylands, any *Podocarpus cunninghamii* vegetation community falling within class six (*Podocarpus cunninghamii* – (*Coprosma propinqua*) treeland) should be considered as highly degraded. This community has lost much of its diversity, having the lowest Shannon diversity score of all the classes (1.90; Table 3.4), and with much of this value comprised by exotic species. It also has a very sparse shrub component, giving it the title of 'treeland'. On the other hand classes four, five and seven, while still being highly degraded, offer more intact examples of *Podocarpus cunninghamii* vegetation communities; as such, working within the constraint of finite resources, they may offer better opportunities for ecological restoration.

4 Arbuscular mycorrhizae and forest restoration on ex-agricultural land

4.1 Introduction

Agricultural systems (croplands and pastures) cover approximately 40% of the Earth's land surface (Foley *et al.*, 2005). The creation of agricultural systems often leads to the destruction of existing ecosystems, and as such agricultural expansion has and continues to be a major driver of global deforestation (Foley *et al.*, 2005, Verchot, 2010). While the conversion of forest to agriculture is continuing apace in the tropics, there is also a global trend of increasing agricultural abandonment (Cramer *et al.*, 2008). The abandonment of agricultural land can allow the return of pre-agricultural flora over extensive areas, which can have positive outcomes for the conservation of indigenous biodiversity (Bowen *et al.*, 2007). However, agricultural practices leave legacies through altered hydrology, fertilisation and tillage, which can prevent the return of desirable native vegetation and allow a degraded community dominated by exotic species to persist into the long term (Cramer *et al.*, 2008). Under such circumstances active ecological restoration is necessary, and therefore an understanding of the processes that affect plant community restoration on abandoned agricultural land is of great importance (Cramer *et al.*, 2008).

The conversion of forest to agriculture can have large impacts on the soil microbial community by causing alterations in community composition and a reduction in overall microbial biomass (Verchot, 2010). The soil microbial community is of fundamental importance to key ecosystem processes such as nutrient cycling, soil development and structure, and plant-soil interactions (Harris, 2009, van der Heijden *et al.*, 2008), and thus any changes to the microbial community can have profound effects on biogeochemical processes (Matson *et al.*, 1997). In light of this, the overall 5">613</key></foreign-keys><ref-type name="Journal Article">17y to depend very heavily on addressing any changes in the microbial community that may have arisen as a result of forest conversion to agriculture and subsequent agricultural practices.

Mycorrhizal fungi are a particularly important component of the microbial community, due to their influence on plant community structure and diversity (Grime *et al.*, 1987, van der Heijden *et al.*, 2008, van der Heijden *et al.*, 1998b, Wardle *et al.*, 2004). Of the mycorrhizae, the arbuscular mycorrhizal fungi (AMF) are by far the most common, forming symbiotic associations with approximately two-thirds of all terrestrial vascular plant species (Smith and Read, 2008). The AMF communities of adjacent agricultural and forest systems has been found to be different (Helgason *et al.*, 1998, Uibopuu *et al.*, 2009), but the implications of these differences for forest restoration on ex-agricultural sites is not yet known. Furthermore, interest in the use of AMF for ecological restoration has been increasing (Renker *et al.*, 2004), and numerous commercially available AMF products are now available. However, commercial AMF isolates are often exotic to the plants and locations where they are applied, and the ecological consequences of their introduction is also not yet known (Schwartz *et al.*, 2006).

This chapter reviews the literature pertaining to the effects of forest conversion to agriculture, and agricultural practices, on AMF communities, as well as the implications this has for forest restoration; and as such it serves as an overall introduction to the following five chapters (Chapter 5 to 9). The review is split into four sections. The first introduces AMF and briefly outlines their role in altering plant relations with the soil environment through nutrient acquisition, soil aggregation, and by providing protection from root pathogens. The second looks at how AMF communities are affected by the conversion of forest to agriculture, by agricultural practices, and what implications this may have for forest restoration. The third examines how pre-inoculation of seedlings with different AMF inocula affects tree and shrub species restoration on ex-agricultural sites. The fourth section draws conclusions on the potential use of AMF for forest restoration on ex-agricultural sites, highlights knowledge gaps, and outlines the structure of the following five chapters.

4.2 Arbuscular mycorrhizal fungi

Several types of mycorrhizal fungi exist. The most common are the AMF, ecto-, ericoid and orchid mycorrhizal fungi. By far the most abundant are the AMF, forming symbioses with an estimated two-thirds of all terrestrial plant species (Smith

and Read, 2008), including angiosperms, gymnosperms and pteridophytes. Despite this near ubiquity there are relatively few described/known species of AMF (approx. 200) worldwide (www.amf-phylogeny.com).

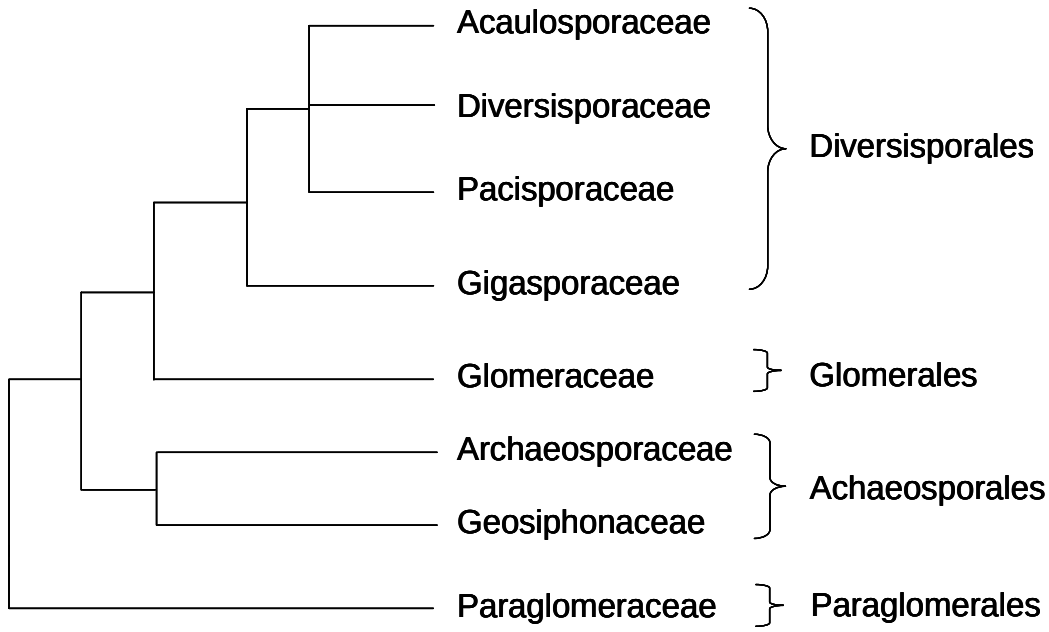

Figure 4.1 – The phylogeny of the Glomeromycota. Simplified from Redecker & Raab (2006).

AMF are a monophyletic group of fungi comprising the orders Archaeosporales, Diversisporales, Glomerales and Paraglomerales that collectively form the phylum Glomeromycota (Schüβler *et al.*, 2001; Figure 4.1). Morphologically, AMF are distinguished from other forms of mycorrhizal fungi by the presence of arbuscules, and in some cases vesicles. Arbuscules only develop within the cortical cells of the host's root and are the fungus' primary functional appendage for nutrient exchange with the plant, particularly of phosphate (Jakobsen *et al.*, 2003). While arbuscules penetrate the cell wall of the cortical cells, they do not penetrate the plasma membrane. The arbuscule, formed by a proliferation of dichotomous branches, invaginates the plasma membrane and thus creates a vast intracellular exchange surface between fungus and plant (Fitter, 1997). Arbuscules are short-lived structures, with an estimated lifespan of between 4-14 days (Alexander *et al.*, 1988). Vesicles, the storage organs that can develop both intra- and intercellularly within the

host root cortex, occur in approximately 80% of described AMF species while arbuscules occur in all (Smith and Read, 2008).

The relationship between AMF and host plant lies along a complex continuum ranging from mutualism to antagonism (Johnson *et al.*, 1997, Klironomos, 2003, Kiers and van der Heijden, 2006). Factors affecting the nature of the symbiosis include morphological and physiological characteristics of the plant and fungus, as well as environmental factors such as the rhizosphere, competition dynamics and trophic interactions (Johnson *et al.*, 1997). To exemplify this, tomato plants inoculated with the AMF *Glomus intraradices* suffered a significant reduction in biomass production compared with those inoculated with *Gigaspora margarita*; shoot dry weight of plants treated with *Glomus intraradices* was also significantly less than that of non-mycorrhizal plants (Facelli *et al.*, 2010). It is therefore important when considering the effect that AMF may have on plant growth or survival to not oversimplify the situation to simply plus or minus AMF.

In general terms, AMF gain from the symbiosis through the acquisition of plant synthesised carbon compounds, which can account for 10-20% of total plant photosynthate (Jakobsen *et al.*, 2003, Peng *et al.*, 1993). It is not fully resolved where this carbon exchange takes place, although either the arbuscules or intercellular hyphae are the most likely sites (Jakobsen *et al.*, 2003). In return, the plant receives inorganic soil nutrients, primarily phosphorous (P), which can help promote increased plant growth, as well as a range of other less well investigated potential benefits, including improved water relations and protection from soil borne pathogens (Fitter, 1991, Smith and Read, 2008). These are now described in more depth.

4.2.1 Nutrient acquisition

The importance of AMF for P acquisition was identified early on (e.g. Baylis, 1967, Daft and Nicholson, 1969, Daft and Nicholson, 1966, Gerdemann, 1964, Hayman and Mosse, 1971, Mosse, 1973, 1959). This early research demonstrated that the extent of root colonisation is dependent upon the concentration of P present in the soil, with an inverse relationship apparent, and that when plants were grown in a low P environment, those that were mycorrhizal were invariably more vigorous than

conspecifics that were not colonised. Furthermore, the growth responses of non-fertilised, mycorrhizal conspecific plants were comparable to that of non-mycorrhizal plants receiving phosphate fertiliser. However, the growth responses of non-inoculated plants to fertiliser with no or very low levels of phosphate did not compare well; growth in such plants was greatly reduced, the plants being consistently outperformed by mycorrhizal plants grown in unfertilised treatments.

More recent work has demonstrated the ability of AMF to acquire nutrients other than inorganic phosphate, such as nitrogen (N) and a range of micronutrient cations. For example, Kothari *et al*. (1990) found that AMF colonisation led to higher host root and shoot concentrations of zinc, and higher root concentrations of copper. AMF are also known to uptake inorganic soil N and transfer this to the plant, leading to higher N levels in plants colonised by AMF compared with those not colonised (Govindarajulu *et al.*, 2005, He *et al.*, 2003, Johansen *et al.*, 1992). There is also evidence that AMF can increase the mobilisation of nutrients from organic sources (Koide and Kabir, 2000, Hodge *et al.*, 2001).

Many studies investigating AMF acquisition of soil nutrients have manipulated individual plants that have been colonised by one or more AMF taxa. When numerous plants grow together, as occurs in nature, the situation is somewhat more complex. AMF are able to colonise an extremely wide range of hosts. As such not only are most of the plants within a community colonised by AMF, but they are also interconnected by extraradical AMF hyphae, thus forming common mycorrhizal networks (Read, 1998). Common mycorrhizal networks can allow the transfer of nutrients between intra- and inter-specific plants (Grime *et al.*, 1987, He *et al.*, 2003, Simard *et al.*, 2003). Such networks may be of ecological significance, for example by assisting the establishment of new seedlings near mature plants, or by facilitating the regeneration of a mixed plant community following disturbance (van der Heijden and Horton, 2009).

4.2.2 Plant-soil water relations

Several studies have demonstrated that AMF are able to improve plant soil water relations, especially during periods of water stress (Allen and Allen, 1986, Al-Karaki

et al., 2004), thus allowing improved plant survival during drought. However the mechanisms by which they do this have proven difficult to elucidate.

Ruiz-Lozano *et al*. (1995) found that plant water stress tolerance was related to both AMF induced physiological changes (e.g. transpiration rates, leaf conductance) in the plant and to nutritional status. Potassium uptake was higher in mycorrhizal plants than in controls; potassium is an important solute controlling stomatal activity in relation to bulk leaf water status, and as such plant responses to water stress are linked to potassium content. Similarly, Wu & Xia (2006) found that mycorrhizal plants had greater ability to adjust their osmotic balance when water stressed compared with non-mycorrhizal plants. Plants inoculated with AMF had higher concentrations of organic solutes, such as sugars and non-structural carbohydrates, and inorganic ions, such as potassium and calcium. By transferring these resources to various tissues at appropriate times, the plants are able to adjust their water potentials to maintain high rates of transpiration and photosynthesis despite low soil moisture values. This topic is reviewed further by Augé (2001).

4.2.3 Soil aggregation and carbon storage

AMF can also plant an important role in the process of soil aggregation. Soil aggregation is important as it improves the structure of soil, thus affecting water storage capacity and aeration, as well as reducing its susceptibility to erosion (Rillig and Mummey, 2006). AMF are able to directly and indirectly influence soil aggregation at three scales: 1) at the plant community scale, by influencing plant community composition, net primary productivity, litter quality and nutrient cycling; 2) at the individual host plant root scale, through physical soil entanglement and by affecting the deposition of root exudates; and 3) at the fungal mycelium scale, through physical entanglement of soil particles and interactions with the soil microbial community (Rillig and Mummey, 2006).

AMF also have a potentially large role to play in the process of carbon storage. As already described, plant synthesised carbon compounds are transferred to AMF as part of the symbiosis. These compounds are utilised by AMF for various functions, but potentially of high importance is the production of the hyphal cell wall components

chitin and glomalin (Driver *et al.*, 2005), a recalcitrant carbohydrate and glycoprotein (Wright and Upadhyaya, 1998), respectively. Despite AMF hyphae having a relatively short 5-6 day lifespan (Staddon *et al.*, 2003), their residual chitin and glomalin components last much longer, with estimated soil residence times of 40-50 years (Zhu and Miller, 2003, Steinberg and Rillig, 2003). Glomalin has also been estimated to account for approximately 4-5% of total soil carbon, which is greater than the total carbon biomass of the soil microbial community (Zhu and Miller, 2003). The accumulation of hyphal residues may therefore represent a significant soil carbon sink

4.2.4 Resistance to soil-borne pathogens and pests

The potential for AMF to reduce the impacts of a wide variety of soil pathogens is well documented (Newsham *et al.*, 1995, Borowicz, 2001, Whipps, 2004). In general, AMF have been found to improve plant performance by reducing the intraradical growth of fungal pathogens (Sikes *et al.*, 2009), though the precise mechanisms behind this remain unclear (Lioussanne *et al.*, 2009). It is possible that direct competition between AMF and pathogen for finite root resources reduces pathogen proliferation (Borowicz, 2001), or it could be that physiological responses increase plant resistance, for example through the modification of root exudates (Lioussanne *et al.*, 2009). Furthermore, AMF inoculated plants have been demonstrated to maintain higher levels of productivity compared to non-inoculated controls while still carrying the same level of pathogen burden (Bleach *et al.*, 2008), although the relationship is dependent on AMF species identity.

In terms of resistance to root pests, such as nematodes, AMF have produced ambiguous results. In a meta-analysis of numerous publications, Borowicz (2001) found that AMF had a detrimental effect on the growth of sedentary nematodes, a conclusion that has recently found more empirical support (Oyekanmi *et al.*, 2007), but actually enhanced the growth of migratory nematodes (Borowicz, 2001).

4.3 Effects of agriculture on AMF communities

The effects of agriculture on AMF communities can be split into two categories. First there is the effect of clearing forest and converting the land use to agriculture, and

second, the effect that different agricultural practices have on AMF communities. These effects and their implications for forest and shrub restoration are now discussed.

4.3.1 Forest conversion to agriculture

Mycorrhizal fungi have strong linkages with the aboveground community and can be a determinant of plant community structure and diversity (Hart *et al.*, 2003, Stampe and Daehler, 2003, van der Heijden *et al.*, 2008, van der Heijden *et al.*, 1998b, Wardle *et al.*, 2004, Yao *et al.*, 2008). As such, strong feedbacks exist between mycorrhizal fungi and the plant community. For example, mycorrhizal fungi can affect plant nutrient uptake and plant community composition, which affects litter quality and subsequent nutrient cycling, as well as the supply of carbon provided by the host. This in turn affects soil nutrient availability as well as plant and fungus development, which further affects plant community dynamics (Wardle *et al.*, 2004). The importance of these linkages is further reinforced as the benefit each plant receives from the mycorrhizal symbiosis is dependent upon the identity of the AMF it is colonised by (Klironomos, 2003). Consequently, the clearance of forest and its subsequent conversion to agriculture is likely to have profound impacts on the AMF community (Verchot, 2010).

The act of clearing forest leads to changes in the biological, chemical and physical landscape. Replacing forest vegetation with arable crops or grass for grazing also completely changes the plant community and eliminates most pre-disturbance host species. These disturbances can lead to changes in both the distribution and abundance of AMF (Abbott and Robson, 1991, Bothe *et al.*, 2010). This being said, some studies have found that, based on spore analysis, clearance of forest for agriculture does not result in any significant changes in AMF species diversity (Johnson and Wedin, 1997, Picone, 2000, Schenck and Kinloch, 1980) or spore density (Johnson and Wedin, 1997, Picone, 2000). However, the proportion of small spores relative to large spores has been observed to be greater in pasture than in forest (Picone, 2000), suggesting that while AMF diversity and spore density may be unchanged, turnover in community composition may occur. More recently, Li *et al.* (2007a, 2007b) found greater AMF spore densities in a grassland-woodland savannah

system that has never been cultivated compared with an arable field, but lowest species diversity within the savannah. Conversely, Bedini *et al*. (2007) found AMF spore density and species richness was greater in a forest compared with a maize monoculture. With regard to grazing systems, AMF community composition of an indigenous forest and grazing pasture were found to be distinct, and forest soil contained a richer suite of AMF species than pasture (Gavito *et al.*, 2008).

While the identification of spores to determine AMF community composition is useful, there are a number of problems with this method (Sanders, 2004). It has been established that spore density does not necessarily translate into the extent of mycorrhizae formation (Abbott and Robson, 1991). In addition, investigation of the spore community provides only a snapshot of those AMF currently sporulating, and does not necessarily identify which AMF are actively colonising plant roots (Clapp *et al.*, 1995, Merryweather and Fitter, 1998). Furthermore, spore identification carries inherent uncertainty because of limitations in the number of distinguishing spore morphological features and the ability of AMF to produce dimorphic spores (Krüger *et al.*, 2009).

Advances in molecular biology now allow species and isolates of AMF to be identified without ambiguity based on their ribosomal DNA (rDNA), and can provide a more accurate picture of the "active" AMF community (Redecker, 2000). Conversely, molecular methods may miss species due their rareness or absence of conserved priming sites (Redecker *et al.*, 2003). Molecular analyses of root samples from forest and arable sites have demonstrated AMF community composition to be very different (Daniell *et al.*, 2001, Helgason *et al.*, 1998, Uibopuu *et al.*, 2009). Members of the Glomeraceae have been found to be particularly abundant within arable sites compared with members of the Acaulosporaceae and Gigasporaceae, while the opposite is true within forest (Daniell *et al.*, 2001, Helgason *et al.*, 1998). Total AMF species richness results have been equivocal; Helgason *et al*. (1998) reported lower species richness within an arable field than in adjacent forest, while Uibopuu *et al*. (2009) found no difference. Overall these results suggest that, despite earlier studies suggesting that forest conversion to agriculture did not cause any differences in the AMF community, forest conversion to agriculture actually has a

marked effect in changing AMF community composition, although this may not have negative impacts on AMF species richness or diversity.

4.3.2 Different agricultural practices

Many agricultural practices, including fertiliser application, tillage, the use of monocultures, and growing non-mycorrhizal crops, can all have negative effects on AMF (Gosling *et al.*, 2006). As such, different agricultural practices have different effects on AMF communities. Addition of P fertiliser has been demonstrated to inhibit both root colonisation and sporulation of certain AMF species, while at the same time lead to increases in sporulation of other AMF species (Sylvia and Schenck, 1983, Kahiluoto *et al.*, 2001). This may partly explain the changes in spore density and composition associated with forest conversion to agriculture. Higher levels of P fertilisation are also associated with lower levels of AMF diversity (Gazey *et al.*, 2004). On the other hand, P fertilisation of P deficient agricultural soils in Kenya was found to have no effect on AMF spore density, diversity or composition (Mathimaran *et al.*, 2007). Addition of P fertiliser has also been shown to negatively affect mycorrhizal function, by reducing the formation of mycorrhizae (Boddington and Dodd, 2000b).

Soil disturbance (e.g. through cultivation) is also known to reduce mycorrhizal function (Jasper *et al.*, 1989b, Jasper *et al.*, 1989a). Reductions in AMF spore density and diversity have both been reported with soil disturbance (Boddington and Dodd, 2000a). Tillage is a common form of agricultural soil disturbance and has been found to lead to alterations in AMF community composition, with members of the Glomeraceae more abundant in tilled soils and non-Glomeraceae more common in untilled soils, but has no effect on overall AMF species diversity (Jansa *et al.*, 2002). This selection of AMF may be influenced by differences in functional traits of the Glomeromycota. The Glomeraceae have been demonstrated to produce extensive intraradical versus extraradical hyphae, while the Gigasporaceae, which tend to be more common in untilled soil (Helgason *et al.*, 1998, Jansa *et al.*, 2002), produce extensive extraradical hyphae (Hart and Reader, 2002, Maherali and Klironomos, 2007), and so may be more sensitive to soil disturbance. Tillage has also been linked to the vertical distribution of AMF, with greatest AMF diversity immediately beneath

ploughing depth (Oehl *et al.*, 2005). The use of monocropping versus crop rotation has also been found to cause a reduction in AMF species diversity and turnover in community composition, with increased loss of Acaulosporaceae and Gigasporaceae (Mathimaran *et al.*, 2007, Oehl *et al.*, 2003).

Intensification of domestic livestock grazing has also been implicated to changes in soil microbial community structure through increased urine deposition (Rooney *et al.*, 2006, Rooney and Clipson, 2009). Negative effects on AMF species richness and diversity have been reported as a result of increased N deposition (Egerton-Warburton and Allen, 2000). However, effects of N fertilisation may be linked with soil P status. N fertilisation of P rich soils has been found to reduce AMF species richness and diversity by causing a shift in community composition towards Glomeraceae dominance and the loss rare species, while N fertilisation in P limited soils has the opposite effect (Egerton-Warburton *et al.*, 2007).

High intensity management inputs associated with conventional farming, i.e. frequent fertiliser application, tillage and monocropping, have been found to have a negative effect on AMF species richness compared with low input, organic agricultural systems (Hijri *et al.*, 2006, Oehl *et al.*, 2003, Verbruggen *et al.*, 2010). In addition, conventional management of agricultural soils appears to show strong selective pressure for Glomeraceae (Helgason *et al.*, 1998, Jansa *et al.*, 2002, Wang *et al.*, 2008). Long term legacy effects of conventional agriculture on AMF community composition are known to occur (Hijri *et al.*, 2006), and it has been suggested that the length of time from forest clearance to agricultural abandonment may influence AMF community composition, with shorter time frames maintaining more of the original forest AMF (Johnson and Wedin, 1997). As such, it seems likely that the intensity of agricultural inputs in combination with time since forest clearance will determine the extent of change in the AMF community.

4.3.3 Implications for restoration

Some studies comparing agricultural and forest soils have found AMF diversity and species richness to be similar (Johnson and Wedin, 1997, Picone, 2000), and therefore concluded that AMF are unlikely to limit forest restoration. These studies considered

AMF species richness, diversity and spore density as measures of AMF community change. As such they overlooked the potential effect of changes in AMF species composition that may have occurred. Other studies investigating AMF potential have found that agriculturally disturbed soils led to reduced AMF infectivity and function (Boddington and Dodd, 2000a, Boerner *et al.*, 1996), especially when compared to infectivity of forest soils (Boerner *et al.*, 1996). However, others have found that forest and agricultural soils have similar levels of AMF propagule availability and infectiveness (Asbjornsen and Montagnini, 1994, Maldonado *et al.*, 2000); thus it is hard to generalise.

Results from a study comparing the effect of agricultural versus forest soil inoculum on tree growth found growth was significantly improved with agricultural inoculum (Fischer *et al.*, 1994). This result is surprising given that agricultural practices, namely soil fertilisation, have been associated with a decline in AMF mutualism (Johnson, 1993, Ryan and Graham, 2002). Fertilisation of plants typically leads to a reduction in root:shoot ratio, as plants invest more in above ground rather than below ground tissues (Hermans *et al.*, 2006). This reduction in resources to plant roots may select for more aggressive AMF that exert a higher carbon drain on their host and are therefore less mutualistic (Graham and Abbott, 2000, Johnson, 1993, Ryan and Graham, 2002).

More recent investigations of tree seedling responses to agricultural and forest AMF communities have found seedlings responded equally well to both inoculum types, despite AMF spore analysis revealing the AMF communities of the two soil types were significantly different (Gavito *et al.*, 2008, Onguene and Kuyper, 2005). A study of herbaceous species found that forest herbs responded equally to both forest and agricultural AMF, but that a herb typically associated with arable systems responded vigorously to agricultural AMF (Uibopuu *et al.*, 2009). These results suggest that while forest species may not show specificity in response to either AMF community, non-target plant species may respond positively to agricultural AMF. In terms of restoration this is important, as nuisance weeds or invasive species with AMF-induced increases in fitness may compete effectively against restoration plantings and thereby inhibit tree seedling establishment (Desprez-Loustau *et al.*, 2007, Wolfe and Klironomos, 2005); or may pervade and persist within a restoration

site and thus prevent floristic convergence with primary forest (Fuchs and Haselwandter, 2008, Gross *et al.*, 2010).

Forests can and do redevelop naturally on abandoned farm land over time, for example in eastern USA and western Europe. However, species diversity and similarity of these regrowth forests compared with primary forests is significantly different and is continuing to diverge over time (Baeten *et al.*, 2010, Vellend, 2004). Site (e.g. duration and intensity of agricultural development) and landscape (e.g. proximity, intactness and size of closest forest remnant) factors play an important role in determining plant species dispersal and establishment within regrowth forests (Baeten *et al.*, 2010, Vellend, 2004). In addition, forest composition may be further influenced by an absence of appropriate mycorrhizal symbionts (Kiers *et al.*, 2000, Klironomos, 2003) and hyphal networks (van der Heijden and Horton, 2009).

4.4 Use of AMF for tree and shrub restoration

It is widely recognised that AMF can play a critically important role in ecological restoration, especially in degraded soils with low mycorrhizal inoculum potential (Bothe *et al.*, 2010, Jayahandran and Fisher, 2008, Jeffries *et al.*, 2003, Khurana and Singh, 2001). Application of AMF in restoration can be achieved either by applying AMF directly into field soils or by pre-inoculating plants prior to their outplanting (Jeffries *et al.*, 2003). Field application of AMF would require large quantities of AMF material to be successful and thus would also require mass production of inocula (Jeffries *et al.*, 2003), which is a process not all species of AMF are suited too (Schwartz *et al.*, 2006). On the other hand, inoculation of seedlings within plant nurseries can be achieved using relatively small quantities of inocula within a controlled environment (Jeffries *et al.*, 2003). As such, inoculation of seedlings with AMF prior to field planting offers a particularly promising avenue for plant restoration (Bothe *et al.*, 2010).

Under glasshouse conditions, inoculation of tree seedlings with indigenous forest AMF has been found to have a beneficial effect on seedling growth, survival and nutrient acquisition (Onguene and Kuyper, 2005, Turjaman *et al.*, 2008, Urgiles *et al.*, 2009). Inoculation with AMF has also been demonstrated to improve tree seedling survival under drought conditions (Zhang *et al.*, 2010). One reason that AMF may

improve seedling drought tolerance is through stimulation of root biomass production (Zhang *et al.*, 2010), although evidence for this is conflicting (Green *et al.*, 2005). Under field conditions, the use of forest AMF has been found to improve forest seedling growth and survival within abandoned agricultural sites (Allen *et al.*, 2003, Allen *et al.*, 2005, Ramos-Zapata *et al.*, 2006). Differential effects of AMF from early and late successional forest on seedling growth have been reported (Allen *et al.*, 2003, Allen *et al.*, 2005, Onguene and Kuyper, 2005). This may be related to differences in AMF species composition of early and late successional forests, individual tree species responses to different AMF, as well as edaphic factors (Allen *et al.*, 2003, Allen *et al.*, 2005, Kiers *et al.*, 2000).

In addition to indigenous forest AMF, numerous commercially available AMF products are now available. For restoration practitioners, using such products can be simpler, less time consuming and cheaper than utilising indigenous populations of AMF, which are often uncharacterised and require collection and culturing. However, experiments comparing the use of indigenous versus exotic AMF for the restoration of shrub species have typically found that native plants show greater growth and nutrient acquisition responses to indigenous AMF than to commercial AMF products (Caravaca *et al.*, 2003, Requena *et al.*, 2001). This suggests that, in general, indigenous AMF offer better prospects for restoration than commercially available products (Fuchs and Haselwandter, 2008, Klironomos, 2003).

4.4.1 Practical considerations

An investigation examining the extent of mycorrhizal colonisation within plant nurseries found that, without intentional addition of mycorrhizae, plant colonisation rates are highly variable (Sylvia *et al.*, 1998). To ensure mycorrhizal colonisation, some nursery practitioners therefore add inoculum. When indigenous inoculum is used, practitioners typically collect leaf litter from existing patches of native forest. However, the collection of leaf litter is unlikely to contain large quantities of infective AMF material. This is because AMF tend to proliferate within soil and access predominantly inorganic, rather than organic, mineral resources (Abbott and Robson, 1991, Smith and Read, 2008). In instances where AMF hyphae have been found colonising organic material it is mostly in the deep, highly decomposed leaf litter in

the vicinity of tree roots, and only to a minor extent on the surface leaf litter (Aristizábal *et al.*, 2004). Thus, while duff will contain AMF material, to capture an ecologically representative sample of the forest AMF community it is necessary to collect material from the rhizosphere (Clapp *et al.*, 1995, Corkidi *et al.*, 2008, Merryweather and Fitter, 1998).

The combination of easily available commercial AMF products and the lack of locally sourced, indigenous AMF cultures means that pre-inoculation of seedlings prior to field planting often occurs with commercial AMF products (Schwartz *et al.*, 2006, Mummey *et al.*, 2009). These products typically include isolates of easily cultured Glomeraceae that are exotic to both the location and plant species to which they are subsequently applied (Mummey *et al.*, 2009, Schwartz *et al.*, 2006). Such inoculation will not necessarily result in improved growth of native plants (Fuchs and Haselwandter, 2008, Klironomos, 2003, Renker *et al.*, 2004). Furthermore, when planted into stressful environments, exotic AMF are not always able to survive or provide plant benefit, as they are not adapted to local conditions (Gianinazzi and Vosátka, 2004, Oliveira *et al.*, 2005). Recent research has also demonstrated that the use of exotic AMF during plant production can have negative effects in realising natural ecosystem AMF diversity after seedlings are planted in the field (Mummey *et al.*, 2009). Finally, the use of exotic AMF necessitates the introduction of non-native species to novel environments. These exotic species have the potential to become invasive, and as the ecological consequences of their introduction is currently not known, caution is advised on their use for ecological restoration (Schwartz *et al.*, 2006).

4.5 Conclusions and knowledge gaps

Research to date shows a general trend of AMF community turnover as a result of forest conversion to agriculture and subsequent agricultural practices. Species of Glomeraceae appear to proliferate while other AMF families decline. While change in AMF community composition may not necessarily have any negative effects on AMF diversity or species richness, it can lead to a reduction in mycorrhizal functioning and mutualism. Given the strong linkages between AMF and the

aboveground community, changes in the AMF community can have major consequences for the plant community, with resultant implications for restoration.

At present little is known of the composition of many of the world's AMF communities and how they interact with the flora present in specific locations. More information is required on how different agricultural practices affect AMF community composition and function. In particular, the majority of research relating to agricultural practices has been conducted on arable sites, with the result that grazing systems and rangelands remain under-studied. Research also suggests that underlying soil fertility, i.e. whether soils are P limited or not, is strongly linked to AMF community responses to fertiliser additions.

In addition to studying the effects of agricultural practices on AMF community structure, further investigation of the implications of changes to AMF community structure for forest restoration are required. Current research indicates that forest AMF will either have no effect or a positive effect on native plant restoration while agricultural AMF, by being more mutualistic with non-target or invasive plant species, may be detrimental. This also applies to the use of exotic, commercially available AMF, as current research shows they are less beneficial to native plants than indigenous AMF.

All of the cited studies pertaining directly to the use of AMF for restoration did not take into account the effect of plant competition. However, when restoring abandoned agricultural land exotic species, particularly pasture species, are common, and can represent a major barrier to forest restoration (Cramer *et al.*, 2008). Furthermore, the outcome of plant competition is influenced by AMF identity (Scheublin *et al.*, 2007). As such, investigation into the effects of AMF from forests versus agroecosystems versus commercial products on the success of forest restoration should also investigate how forest plant species respond to competition. Specifically, research should investigate how they respond to competition with those plant species that limit restoration success within their particular geography.

4.5.1 Outline of next five chapters

The next five chapters (Chapters 5 to 9) explore the potential role of AMF in the restoration of *Podocarpus cunninghamii*. Chapter 5 is a molecular investigation aimed at identifying the species that comprise the AMF communities that colonise *Podocarpus cunninghamii* in the high country. In addition, Chapter 5 investigates AMF community composition of grasslands adjacent to existing *Podocarpus cunninghamii* stands, to elucidate changes in AMF community composition that may have occurred since forest conversion to grazing grassland. Chapter 6 investigates the effect that AMF communities isolated from a remnant high country stand of *Podocarpus cunninghamii* and a retired grazing grassland have on the growth and nutrient acquisition of *Podocarpus cunninghamii* cuttings. Chapter 7 compares the growth and nutrient responses of *Podocarpus cunninghamii* cuttings to an exotic, commercially available AMF product and an indigenous, single species AMF culture. Both Chapters 6 and 7 are glasshouse based. They both also investigate how competition with *Agrostis capillaris*, an exotic grass species that is invasive within the high country, affects the response of *Podocarpus cunninghamii* to the different AMF treatments. Chapter 8 reports on the results of a high country field trial investigating the effects of all the different AMF inocula on the growth of *Podocarpus cunninghamii*. Finally, Chapter 9 investigates if and how AMF are used within native plant nurseries, to identify whether the potential benefits for restoration that can be gained by inoculating seedlings with AMF are being realised.

5 Investigation of high country *Podocarpus cunninghamii* and grassland AMF communities

5.1 Introduction

The composition of AMF communities in forest and agricultural systems has been shown to be very different (Helgason *et al.*, 1998, Uibopuu *et al.*, 2009). This difference may represent a constraint to the restoration of forest on ex-agricultural land through the loss of AMF function and mutualism, as well as specific AMF symbionts (see Chapter 4). In New Zealand, no work has been done to identify if any such differences exist.

Before the last decade, identification of AMF taxa relied primarily upon morphological identification of spores (Moorman and Reeves, 1979, Schenck and Pérez, 1990). This method is unreliable for a number of reasons, including the limitation in number of distinguishing morphological characters and the ability of AMF to produce dimorphic spores (Krüger *et al.*, 2009, Sanders, 2004). Furthermore, investigation of the spore community provides only a snapshot of those AMF currently sporulating, and does not necessarily identify which AMF are actively colonising plant roots (Clapp *et al.*, 1995, Merryweather and Fitter, 1998). Advances in molecular biology have allowed species and isolates of AMF to be identified without ambiguity and analysed to investigate their degree of similarity, based on their ribosomal DNA (rDNA). Molecular analysis can thus provide a more accurate overall picture of the AMF community (Redecker *et al.*, 2003).

The aims of this chapter were to 1) confirm that *Podocarpus cunninghamii*, irrespective of whether it is growing in areas of extensive intact forest in regions of high rainfall or small, scattered remnant stands within the dry, predominantly agricultural hinterland, does naturally form AMF associations; 2) determine the identity of AMF forming these associations using molecular methods targeting the small subunit (SSU, 18S) and large subunit (LSU, 28S) rDNA regions; and 3) similarly determine the identity of AMF in grasslands adjacent to the sampled stands

of *Podocarpus cunninghamii*, to elucidate any differences in the composition of the AMF communities between the vegetation types.

5.2 Methods

5.2.1 Collection of root material

Podocarpus cunninghamii root samples were collected from five high country sites. These sites followed a west-east rainfall gradient, from Mount Cook National Park (>2,500 mm annual rainfall), to the southern end of the Mackenzie Country near Omarama (<600 mm annual rainfall). The high country does not experience a marked wet or dry season, thus annual rainfall is distributed evenly throughout the year. Two sites of opposite aspect were selected from Birdwood Station (NBS (north aspect) and SBS (south aspect)), one site from Longslip Station (LS), one from Mt Cook National Park (MC), and one from Mt Ben Ohau (MB); for precise site locations and descriptions of each site see Chapters 3. Eight root samples were taken from each site. Attempts were made to collect two samples per sampled tree (north and south aspect) however with the rockiness of the sites this was not always possible. In such instances the second sample was taken from the nearest neighbouring tree. A total of 40 samples were collected, stored in plastic bags in a cool box, and transported to the School of Forestry, Christchurch. Samples were then stored in a refrigerator at 4ºC. Samples were collected in June 2008 and again in February 2010.

Root material was also collected from three grassland sites: Balmoral Station (BA; 44° 02'S, 170° 24'E), and two grassland sites adjacent to the *Podocarpus cunninghamii* stands at Mt Ben Ohau and Mt Cook National Park. These grasslands were depleted *Festuca novae-zelandiae* tussock grasslands, heavily invaded by *Agrostis capillaris, Anthoxanthum odoratum* and *Hieracium pilosella*, with these invading species comprising approximately 75% of the vegetation cover. Samples were air-dried and stored at 4ºC. Only molecular analysis was applied to these samples.

5.2.2 Preparation of root material

Podocarpus cunninghamii roots are woody, and as such often darkly pigmented. To overcome this pigmentation, five preparation methods were trialled before a final

methodology was selected. For all methods, each root sample was first rinsed with water to remove excess soil and the roots of other species. A subsample from each tree was then taken for further preparation.

Method One

Roots were cleared of pigment by being soaked in 10% (w/v) KOH for 2.5 hours in an 80°C water bath. The roots were then rinsed with reverse osmosis (RO) water before being neutralised in 10% (v/v) HCl and stained overnight in 0.05% (w/v) trypan blue solution.

Method Two

Roots were soaked in 10% (w/v) KOH for 30 minutes in a 90°C water bath before being rinsed, neutralised and stained as per Method One.

Method Three

Roots were soaked in 10% (w/v) KOH as per Method One, but after rinsing with RO water were bleached in 3% (v/v) H_2O_2 for 5-20 minutes (depending on the level of pigmentation) at 85°C. They were then rinsed with water and soaked in 1% (v/v) HCl for 10 minutes. Finally they were stained overnight in 0.05% (w/v) trypan blue solution.

Method Four

Roots were soaked in 10% (w/v) KOH for 30 minutes in a 90°C water bath, rinsed with RO water, and bleached and stained as per Method Three.

Method Five

Roots were soaked in 10% (w/v) KOH and placed in an 80ºC water bath for four hours. These roots were then rinsed, bleached and stained as per Method Three.

For all methods, the stained roots were stored in lactic glycerol (50% (v/v) glycerol, 25% (v/v) lactic acid, 25% sterile distilled water) (Brundrett *et al.*, 1996, Redecker *et al.*, 2003). Method Five gave the best results, in terms of pigment-cleared roots, and so was the method adopted for the preparation of all samples for analysis of root colonisation.

The remainder of the roots were then used to initiate arbuscular mycorrhiza spore cultures (section 5.2.5).

5.2.3 Percentage root length colonised

The gridline intersection method (Giovannetti and Mosse, 1980) was used to visually confirm the presence and determine the extent of AMF colonisation on the primary root cortex. A grid (1 cm^2 grid square) was etched onto an 8.5 cm diameter Petri dish and 1 cm root pieces were randomly distributed. These were observed under a dissection microscope at ×80 magnification both horizontally and vertically; a minimum of 100 intersections was evaluated for each sample. The number of intersections observed was recorded, as too was the number of times AMF features intersected; this provided percent colonisation. The length of root analysed (L) was then calculated using the following equation (Brundrett *et al.*, 1996):

$$L = N \times G \times 11/14 \qquad \text{(Equation 5.1)}$$

Where N is the number of intersects and G is the length of one side of a grid square, in this case 1 cm. This value was then multiplied by the percent colonisation to give the length of root colonised.

Percentage root colonisation data was arc-sine square root transformed prior to analysis using a two-way analysis of variance (ANOVA), with collection site and aspect set as factors. Significant differences between factor levels were identified through stepwise model simplification (Crawley, 2007). Analysis was conducted using R version 2.10.0 (R Development Core Team, 2009).

5.2.4 Molecular methods with root material

Ten *Podocarpus cunninghamii* and four grassland root samples were initially selected to optimise the various molecular methods before adopting a specific protocol for use across all samples.

5.2.4.1 DNA extraction

Three extraction methods were trialled in order to find the technique that yielded the greatest quantity and quality of DNA. The first method (Method A) used Chelex-100™ (Bio-Rad Laboratories) in a method modified from Ishii & Loynachan (2004). The second method (Method B) utilised the REDExtract-N-Amp Kit™ (Sigma Aldrich Inc.). The third (Method C) used the Puregene® DNA Isolation Kit (Gentra Systems). For all methods, colonised roots were thrice washed in sterile distilled water and surface sterilised with 5% (w/v) chloramine-T hydrate (Sigma-Aldrich Inc.). They were then crushed in liquid nitrogen using a mortar and pestle prior to extraction by methods A, B and C.

Method A

Crushed root material was placed in 60 µl of 10% (w/v) Chelex-100 and incubated for 10 minutes at 90°C. Samples were then placed on ice for 1 minute before being centrifuged for 5 minutes at 12,000 × *g*. The supernatant was removed and stored at -20ºC until use.

Method B

Crushed root material was placed in 100 µl of REDExtract-N-Amp™ Extraction Solution and incubated for 10 minutes at 95°C. One hundred µl of REDExtract-N-Amp™ Dilution Solution was then added and the mixture vortexed. The final solution was stored at 4°C until use.

Method C

Crushed root material was placed in 500 µl of Cell Lysis solution and incubated at 65ºC for 60 minutes. Each sample was inverted 10× after 30 and 60 minutes. After cooling to room temperature, 167 µl Protein Precipitation solution was added and the samples vortexed for 20 seconds, followed by centrifugation at 14,000 × *g* for 3 minutes. The supernatant (containing the DNA) was transferred to a clean tube and the DNA was precipitated by the addition of 500 µl of isopropanol. Each sample was inverted 50× to mix then centrifuged at 14,000 × *g* for one minute. The supernatant was removed and each tube left to drain. The DNA pellet was washed by the addition of 300 µl of 70% ethanol and inverted several times, then centrifuged at 14,000 × *g* for 1 minute. The ethanol was poured off and the tubes left to drain and air dry for

10-15 minutes. Subsequently, 50 µl DNA Hydration solution was added and the samples incubated at 65ºC for 60 minutes. Tubes were tapped periodically to disperse DNA. After incubation the tubes were centrifuged at 14,000 × *g* for five minutes and the supernatant (containing the DNA) transferred to a clean tube.

For each method, 1.5 µl of each DNA extract was assessed for concentration and purity by spectrophotometry (NanoDrop 3.0.0, Thermo Scientific). Samples were diluted with sterile distilled water where necessary to achieve a DNA concentration of approximately 20 ng. μl^{-1}. Methods A and B were trialled over the period July 2008 to May 2009; Method C was trialled during February 2010.

5.2.4.2 Polymerase chain reaction (PCR)

5.2.4.2.1 Reagent mixtures

Method A

One µl of DNA template was added to 19 µl of PCR mixture consisting of 1× PCR reaction buffer (50 mM Tris/HCl, 10 mM KCl, 5 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, pH 8.3/25°C), 200 µM of each dNTP, 1 U FastStart *Taq* polymerase (Roche) and 5 pmoles of each forward and reverse primers (Table 5.1).

Method B

Four µl of DNA template was added to 16 µl of PCR mixture consisting of 10 µl REDExtract-N-Amp™ Ready Mix (Sigma-Aldrich Inc.), 4 µl sterile distilled water, and 5 pmoles of each of the same primers.

Method C

Two PCR amplification mixtures were trialled. Either 1 µl or 3 µl of DNA template were added to 19 µl or 17 µl of PCR mixture, respectively; the PCR mixture was that used for amplification of DNA extracted using Method A, making adjustments for the quantity of sterile distilled water. Where the Krüger *et al.* (2009) primer mixes were used, the quantity of sterile distilled water was reduced to maintain a final PCR mix of 20 µl.

AMF specific primers designed for the small subunit (SSU, 18S) and large subunit (LSU, 28S) ribosomal DNA (rDNA) regions were used in combination with more

general eukaryotic primers (Figure 5.1); the primer combinations used are listed in Table 5.1.

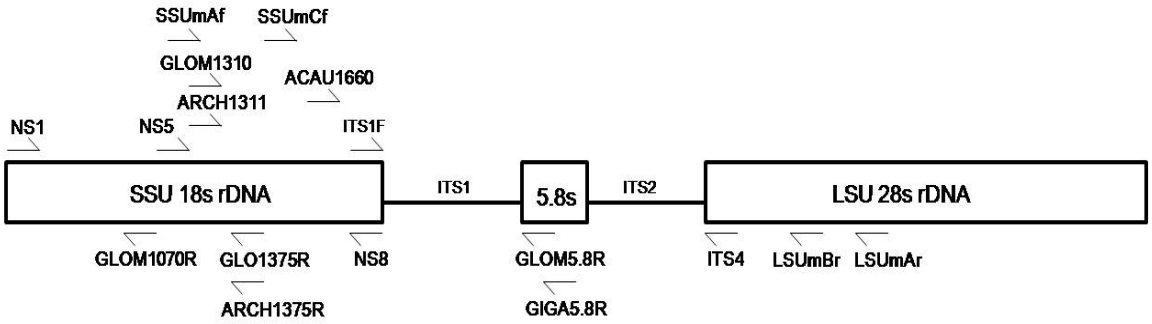

Figure 5.1 – The relative location of the forward and reverse primers used in the study. Diagram is not to scale. Adapted from Russell *et al.* (2002) and Krüger *et al.* (2009).

5.2.4.2.2 Amplification conditions for standard and nested PCR

Methods A and B

A modified version of the thermal cycle used by Redecker (2000) was used for PCR: a 4 minute denaturation step at 94°C, followed by five cycles of 94°C for 30 seconds, 61°C for 30 seconds (annealing phase) and 72°C for one minute (extension phase). Following this they were amplified by a further 30 cycles with an annealing temperature of 60°C. The final extension phase lasted for 10 minutes before being held at 4°C. All PCRs were done using a Bio-Rad iCycler™.

Two forms of nested PCR were also trialled – hemi-nested and fully nested. Three different primary PCRs were done; one using the universal forward and reverse primers NS5 and ITS4, one using NS1 and ITS4, and one using SSUmAf and LSUmAr. The full primer combinations and their source are listed in Table 5.1.

The primary and nested PCRs followed the methodology of Redecker (2000). Where gel bands were present following 1% agarose gel electrophoresis of a 5 µl aliquot of the primary PCR (see 5.2.4.2.3 for method), the PCR products were diluted 1:1000 in nuclease free water; where no gel bands were present the products were diluted 1:100. One µl of diluted primary PCR product was then added to 19 µl of PCR mixture consisting of 1× PCR reaction buffer (50 mM Tris/HCl, 10mM KCl, 5 mM

$(NH_4)_2SO_4$, 2 mM $MgCl_2$, pH 8.3/25°C), 200 µM of each dNTP, 1 U FastStart *Taq* polymerase (Roche) and 5 pmoles each of the respective primer combinations (Table 5.1). The annealing temperature for the primary PCRs was 51°C for 35 cycles while the annealing temperatures for the nested PCRs were 61°C for five cycles followed by 60°C for 28 cycles.

Table 5.1 – Primer combinations for the non-nested, hemi- and fully nested PCRs. AMF specific primers are from (*) Redecker (2000), (**) Redecker *et al*. (2000), (†) Russell *et al* (2002), (‡) Krüger *et al.* (2009). ITS1F is from Gardes & Bruns (1993); all others are from White *et al.* (1990).

Type of PCR	**Primary PCR primers** (forward – reverse)	**Secondary PCR forward primer**	**Secondary PCR reverse primer**
Non-nested	ITS1F – GIGA5.8R*		
	ACAU1660* – ITS4		
	GLOM1310* – NS8		
	NS1 – ARCH1375R†		
	SSUmAf‡ – LSUmAr‡		
Hemi-nested	NS5 – ITS4	ACAU1660*	ITS4
		ARCH1311*	
		GLOM1310*	
		NS5	GIGA5.8R*
			GLOM5.8R*
Fully nested	NS5 – ITS4	ARCH1311*	GIGA5.8R*
		GLOM1310*	
		ARCH1311*	GLOM5.8R*
		GLOM1310*	
	NS1 – ITS4	NS3	ARCH1375R†
			GLO1375R†
			GLOM1070R (**)
	SSUmAf‡ – LSUmAr‡	SSUmCf‡	LSUmBr‡

Method C

The primary and nested PCR protocols followed Krüger *et al.* (2009). For primary PCR initial denaturation was 5 minutes at 99°C, followed by 40 cycles of 10 seconds at 99°C, 30 seconds annealing at 60°C and one minute elongation at 72°C; final elongation was 72°C for 10 minutes. Nested PCRs were the same except that the

annealing temperature was 63°C and for only 30 cycles. Primary PCR products were diluted 1:10 for use in nested PCR.

5.2.4.2.3 Agarose gel electrophoresis and DNA sequencing

For Methods A and C, and the nested PCR products, 10 µl were mixed with 2 µl of loading buffer (0.025% (w/v) bromophenol blue, 0.025% (w/v) xylene cyanol, 40% (w/v) sucrose in water). REDExtract-N-Amp™ Ready Mix PCR products (Method B) do not require the addition of a loading buffer. Ten µl of all PCR products were run on 1% agarose gel submerged in 1× TAE (40 mM Tris Base, 20 mM acetate, 2 mM EDTA, pH 8) for 50 minutes at 10 $V \cdot cm^{-1}$). A molecular marker was run concurrently by mixing the loading buffer with 5 µl of 1 Kb plus DNA Ladder™ (Invitrogen). Gels were stained by soaking in 0.5 $\mu g.ml^{-1}$ ethidium bromide solution for 15 minutes, followed by de-staining in water for five minutes. Gels were the photographed under ultra violet light using the VersaDoc™ imaging system (Rio-Rad).

Where multiple bands were present or the amplimer was faint, the purity/concentration of the gel bands from the ACAU1660–ITS4 nested PCR were extracted directly from the agarose gel by stabbing the gels with a sterile pipette tip. Each extracted product was then placed in 19 µl of PCR reaction mixture and subjected to the nested PCR cycle.

All samples producing bands were sequenced using a 3100-*Avant* Genetic Analyzer (Applied Biosystems) and analysed using Sequencing Analysis Software version 5.1 (Applied Biosystems). All unambiguous returned sequences were queried on GenBank using the Basic Local Alignment Search Tool (BLAST) (Zhang *et al.*, 2000) for known matches. The neighbour joining algorithm, with the maximum allowable fraction of mismatched bases set at the BLAST default of 0.75, were then used to identify which clade the sequence belonged. These sequences (phylotypes) were named according to the major genus clade in which they appeared; the first four letters of the genus followed by a numerical index, *x*. For example, *Glomus* phylotypes were named Glom-*x*.

To place our phylotypes within a New Zealand podocarp AMF context they were aligned with the sequences published by Russell *et al*. (2002) for podocarps growing on the west coast. The phylogenetic relationship between phylotypes was then derived using the neighbour joining method with 1,000 bootstrap replicates. Sequence alignment, phylogenetic analysis and tree construction was all done using Mega 4.1 (Tamura *et al.*, 2007).

5.2.4.3 Resolution of mixed PCR products by SSCP and cloning

Two methods were utilised to separate PCR products containing multiple sequences in the final nested PCR mixtures. These were single stranded conformation polymorphism (SSCP) and TA cloning. The products from these two methods were then sequenced as described in section 5.2.4.2.3.

Single stranded conformation polymorphism (SSCP)

All the nested PCR products that produced bands during agarose gel electrophoresis were used for SSCP. For a review of the underlying molecular biology upon which SSCP is based, and the applications therein of SSCP, see Kerr & Curran (1996).

A non-denaturing 0.625× MDE™ (Lonza) polyacrylamide gel was made using 12.5 ml of 2× MDE™ gel solution (Lonza) with 8 ml of 5× TBE (445 mM Tris Base, 445 mM borate, 10 mM EDTA, pH 8) and 19.5 ml of H_2O. Following this 30 µl of N, N, N', N'-tetra-methyl-ethylenediamine (TEMED, Bio-Rad Laboratories) and 280 µl of 10% (w/v) ammonium persulphate (Bio-Rad) were added. The solution was mixed, loaded into an assembled vertical Protean® II xi Cell (Bio-Rad) and left for 2 hours to polymerise. Once polymerised, the acrylamide gels were submerged in 1× TBE buffer in the Protean® II xi Cell gel tank.

For PCR products that produced strong bands in agarose gel electrophoresis, 1.5 µl of PCR product was mixed with 15 µl of loading buffer (95% (v/v) formamide, 0.05% (w/v) bromophenol blue, 0.05% (w/v) xylene cyanol); for those that produced only weak bands, 3 µl of product was mixed with 15 µl of loading buffer.

These prepared samples were incubated at 95°C for 5 minutes, to denature the template strands, immediately cooled on ice for 5 minutes, then loaded into the wells on the acrylamide gel. Electrophoresis was run for 17 hours at 200 V using a cooling system that maintained the running gels at 20°C. This method was modified from Schwieger & Tebbe (2000).

Gels were then silver stained in a glass tray. First, each gel was placed in fixer solution (10% (v/v) ethanol, 0.5% (v/v) acetic acid) and rocked for 5 minutes. This solution was then replaced with a staining solution (10% (v/v) ethanol, 0.5% (v/v) acetic acid, 0.2% (w/v) $AgNO_3$). Gels were stained for 5 minutes, washed with dH_2O before being covered in developer solution (3% (w/v) NaOH, 0.1% (v/v) formaldehyde) for 30 minutes.

Post-staining, both the gels were sandwiched between two acetate sheets and scanned. Clear bands were excised from each of the gels using a scalpel blade and forceps, both sterilised with 70% ethanol between gel excisions. Gel slices were stored in 100 µl of dH_2O at -20°C.

Three methods were employed to extract and amplify the DNA from the acrylamide gel slices. The first involved cutting a 2 mm^2 section of gel slice and placing it within 19 µl of PCR reaction mixture. The second was identical except that the 2 mm^2 gel fragment was masticated using sterile forceps. The third method used a freeze-squeeze procedure, modified from Tautz & Renz (1983). This method was developed for DNA extraction from agarose gel but is also commonly used for extraction from acrylamide gels (Kurien and Scofield, 2002). In this method, a fine hole (approximately 2 mm diameter) was punched through the base of a 0.6 ml tube. A small quantity of glass wool was then firmed down above this hole and the gel slice placed atop. This tube was then wrapped in aluminium foil and frozen in liquid N_2. Once frozen, the 0.6 ml tube was placed within a 1.7 ml tube and centrifuged at 16,100 × *g*. The centrifugation process forced DNA fragments through to the base of the 0.6 ml tube and into the 1.7 ml tube, while the glass wool retained the gel within the 0.6 ml tube. One µl of the resulting DNA solution was then added to 19 µl of PCR mixture. For all three methods, the PCR protocol had an annealing temperature of 60ºC and ran for 40 cycles, as described in section 5.2.4.2.2.

PCR products were run on a 1% agarose gel following the procedure given in section 5.2.4.2.3. Samples that produced clear, single bands were selected for sequencing.

Cloning

Four site samples were selected at random for cloning (two from Mt Cook National Park and one from each of Birdwood Station (north) and Mt Ben Ohau); samples were ligated overnight at 4ºC. The 10 µl ligation mixture consisted of 5 µl of 2× Rapid Ligation Buffer for T4 DNA ligase, 1 µl of pGEM®-T Easy vector (50 ng) (Promega), 1 µl of PCR product, 1 µl T4 DNA ligase (3 Weiss units per µl), and 2 µl sterile distilled water.

Two solutions (lysogeny broth (LB broth) and LB media plus agar) were made, each using the following ingredients: 10 g Bacto-tryptone, 10 g NaCl, and 5 g Bact-yeast extract; 15 g of agar was added to the LB media plus agar. Sterile distilled water was used to make the solutions up to 1 litre. After thorough mixing the two solutions were sterilised by placing in a pressure cooker for 15 minutes. The LB broth was allowed to cool at room temperature while the LB media plus agar was placed in a 50ºC incubator for 30 minutes. After 30 minutes 1 ml of sterilised ampicillin (100 $\mu g \cdot ml^{-1}$) was added. The ampicillin had been sterilised by filtration through a Millex-GS syringe driven filter unit (0.22 µm) (Millipore). Aliquots of approximately 30-35 ml of LB media plus agar were poured into 85 mm Petri dishes and allowed to solidify.

An X-gal solution (50 mg 5-bromo-4-chloro-3-indolyl-β-D-galactoside in N, N' – dimethylformamide$\cdot ml^{-1}$) and 100 mM IPTG (isopropyl β-D-1-thiogalactopyranoside) was then prepared. Twenty-five µl of X-gal and 50 µl of IPTG were spread, using a metallic spreader that had been sterilised by flaming with ethanol, over each of the solidified LB media plus agar dishes; these dishes are hereafter referred to as LB/X-gal/IPTG/Ampicillin plates.

Two µl of each ligation mixture was added to a sterile 1.7 ml tube on ice. DH5α competent cells (*Escherichia coli* strain DH5α, Promega) were then taken from -80ºC storage and thawed on ice for approximately 5 minutes. The cells were mixed by gently flicking the base of the tube. Fifty µl of thawed cells were then added to each

2 µl aliquot of ligation mixture, the tubes gently flicked to mix and put back on ice for 5 minutes. The cells were heat-shocked for 50 seconds in a 42ºC water bath before being placed back on ice for a further 2 minutes. The cells were allowed to recover by the addition of 250 µl of SOC medium (20 g Bacto-tryptone, 5 g Bacto-yeast extract, 0.5 g NaCl, 2.5 ml 1M KCl, made up to 1 litre with deionised water) and incubation for 1 hour at 37ºC on a shaker.

The transformed cells were plated out onto the prepared LB/X-gal/IPTG/Ampicillin plates at two different cell volumes, one using 100 µl of transformed cells and one using 200 µl. Cells were spread using a metal spreader that was sterilised between uses by ethanol and flaming. The plates were incubated at 37ºC for 24 hours.

White colonies were selected from the plates and used for colony PCR. In this process, a sterile pipette tip is used to collect a small part of colony and is then placed in the PCR reagent mix. The PCR protocol was 94ºC for 2 minutes followed by 35 cycles of 94ºC for 30 seconds, 50ºC for 30 seconds and 72ºC for 45 seconds; a final extension phase ran at 72ºC for 7 minutes. The primers used were SP6 (5' – GATTTAGGTGACACTATAG – 3') and T7 (5' – TAATACGACTCACTATAGGG – 3'), which are specific for sites on the p-GEM®-T Easy vector. PCR products were separated by electrophoresis on a 1% agarose gel. Samples that produced clear, single bands were selected for sequencing. Where multiple samples from a site produced same size bands, one was selected at random for sequencing.

5.2.5 AMF spore cultures

Roots *Podocarpus cunninghamii* of the same aspect at each site were amalgamated to form a total of 10 inoculants (north and south aspects across five sites). Pots of 2.5 L volume were filled to two-thirds with a rooting medium consisting of 50% sand, 30% pumice and 20% low-phosphorous potting mix, all of which had been autoclaved at 121ºC for 1 hour to sterilise. Each pot was then inoculated with root segments before being filled with rooting medium. Water (250 ml) was added to each pot and seeds of white clover (*Trifolium repens* cv Huia) were sown to provide a host plant for the mycorrhizal fungi to colonise. Two control pots, free of mycorrhizal spores or hyphae, were also sown. Spore cultures were initiated in July 2008.

A 100 ml microbial wash was also added before the pots were placed in a glasshouse. The microbial wash was prepared by collecting a sample of woodland soil and sieving it to 2 mm. Ten % (w/v) of soil was placed in water and stirred for 2 hours. The mixture was left to settle for 4 hours before being suction filtered through progressively finer grades of filter paper (20-25 µm > 11 µm > 6 µm). The final grade is fine enough to remove all AMF spores and hyphae. The filtrate contains bacteria, yeast and some soil-borne fungi but does not contain larger organisms such as nematodes and protozoa.

5.2.5.1 Spore extraction and species identification

The cultures were checked periodically for spore abundance by extracting soil cores from randomly selected pots in August and November 2008, and in January 2009. In January 2009 all above ground clover material was removed and the rooting medium, with roots intact, left to air dry. Once air dried, all samples were bagged and stored at 10°C.

One hundred g samples were taken from each culture and spores extracted by wet-sieving; the 100 g sample was soaked in water for 1 hour, being stirred vigorously every 20 minutes. The aqueous portion was then poured through successively finer sieves (500 µm > 150 µm > 50 µm). The material that collected on the 150 µm and 50 µm sieves was then centrifuged in 50 ml 50% sucrose solution at 1000 × *g* for 1 minute. The supernatant was then re-sieved at 50 µm before being transferred to Whatman grade 1 (20-25 µm pores) filter paper and viewed under a binocular dissecting microscope (Brundrett *et al.*, 1996).

The spores were separated to genus level according to morphological traits (Schenck and Pérez, 1990). Each spore was surface sterilised by being soaked in chloramine-T hydrate (5% w/v) for 1 minute then washed in sterile distilled water for a further minute; this was repeated three times. Spore DNA was extracted, amplified and sequenced. DNA was extracted by crushing spores and using the REDExtract-N-Amp™ Kit. When one or two spores were crushed, 10 µl each of the extraction and dilution solutions were used; when between three and five spores were crushed, 15 µl each of the extraction and dilution solutions were used. The PCR procedure utilised

the universal ITS4 and fungi specific ITS1F primers, with an annealing temperature of 55° for 40 cycles. A PCR optimisation process, using different numbers of known spores (from single species cultures), different quantities of extraction and dilution solutions, and altering the annealing temperature and number of cycles of the PCR procedure, found the above protocol to be the most reliable in producing clear bands after electrophoresis (H.J. Ridgway, pers. comm.). DNA was also extracted from pieces of clover roots from each culture using the REDExtract-N-Amp™ (Sigma Aldrich Inc.) protocol detailed in section 5.2.4.1.

As the above primers are not AMF specific, it was deemed desirable to use an AMF specific primer for sequencing. This minimised the impact of any non-target DNA that may have been present in the PCR mix, thereby producing cleaner sequence data. Therefore, for the sequencing reaction, AMF specific internal primers were used. For those with *Acaulospora*/*Glomus* morphology, primer GLOM5.8R was used, for those appearing to be *Gigaspora*/*Scutellospora*, primer GIGA5.8R was used. Sequence chromatograms were inspected visually and lengths at the start and end of the sequence that had low signal intensity or were otherwise ambiguous were removed. The remaining sequence lengths were then queried on GenBank using BLAST. Sequences were assigned phylotype names following the procedure described above. These were included in the phylogenetic analysis (section 5.2.4.2.3) to place them in a New Zealand AMF context.

5.3 Results

5.3.1 Preparation of root material

Of the five different clearing methods trialled, Method Five proved to be most effective at removing root pigment and thus revealing mycorrhizal structures. Method Two proved the least effective, with stained roots remaining highly pigmented. Plate 5.1 shows the results from Method's One, Three, Four and Five.

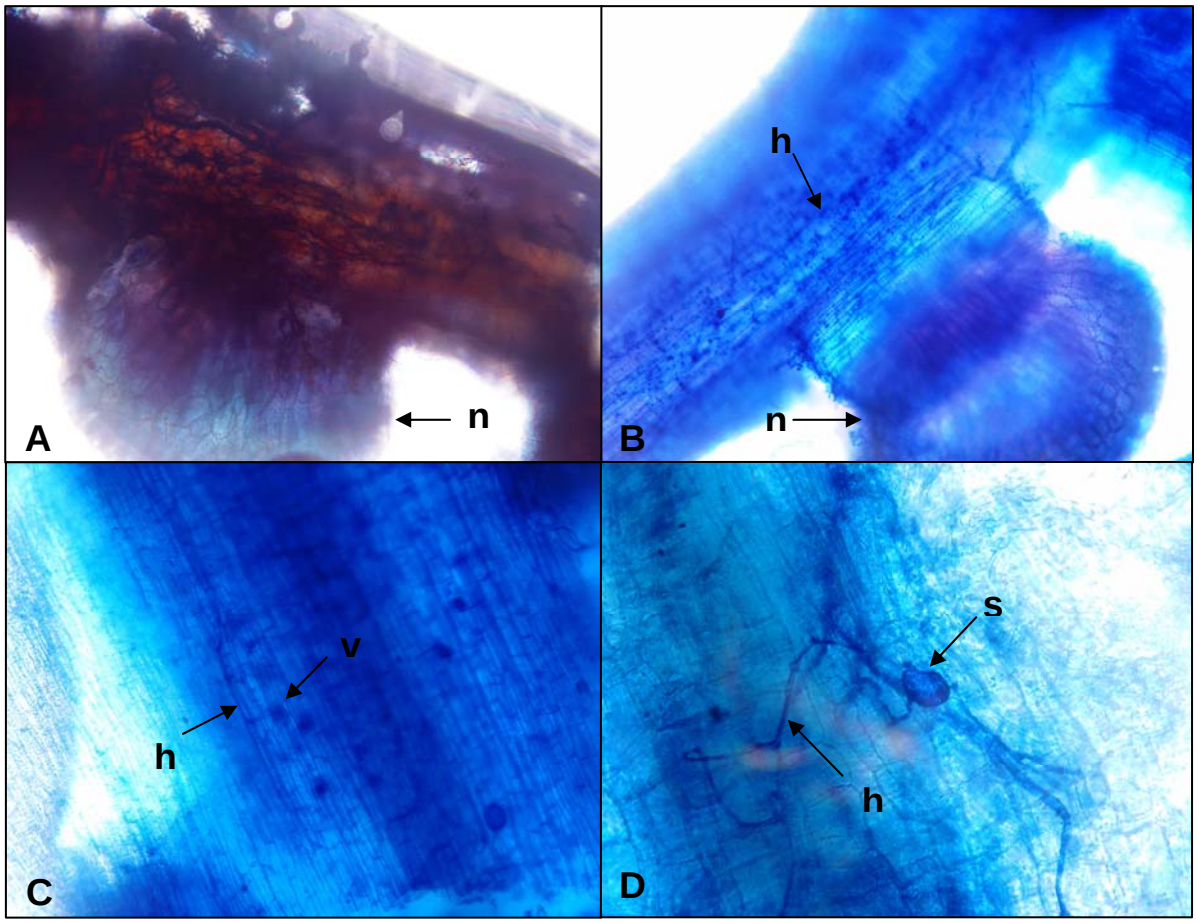

Plate 5.1 – The relative efficacy of four different methods for clearing pigment from fine roots of *Podocarpus cunninghamii*. The labels identify features of note: n = *Podocarpus cunninghamii* root nodule, h = mycorrhizal hyphae, v = vesicle, s = spore. A) Method One (×100 magnification); B) Method Three (×100); C) Method Four (×100); D) Method Five (×400).

5.3.2 Percentage root length colonised

All subsamples from each site were colonised by AMF, as they displayed features characteristic of these fungi, including arbuscules and vesicles (Plate 5.2). The level of colonisation in the sampled trees varied across sites, ranging from 25% at Mt Ben Ohau to 45% at Birdwood Station. Two-way ANOVA found there to be a significant difference between sites, with trees at Birdwood Station (north) and Mt Cook National Park having approximately 20% more root length colonised than the other sites ($F_{1,38}$ = 11.87, $p < 0.01$; Figure 5.2). Aspect had no effect ($F_{1,30}$ = 0.02, $p > 0.8$) and there was no site × aspect interaction ($F_{4,30}$ = 1.19, $p > 0.3$).

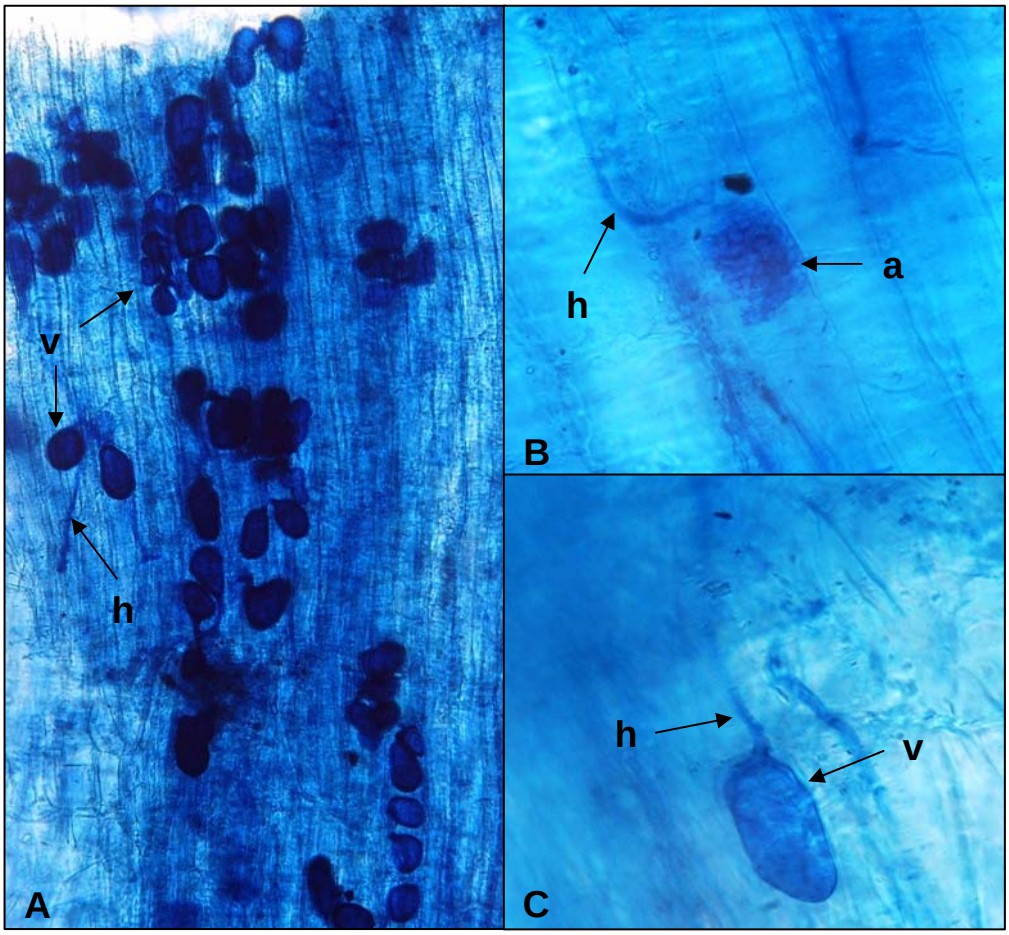

Plate 5.2 – AMF structures observed with trypan blue stained *Podocarpus cunninghamii* roots. A) Irregularly lobed vesicles (×100 magnification); B) Arbuscule with adjoining hypha (×400); C) Irregularly lobed vesicle with adjoining hypha (×400).

5.3.3 Molecular results with root material

5.3.3.1 DNA amplification

None of the grassland samples yielded PCR products in either standard or nested PCR. For all *Podocarpus cunninghamii* samples, standard PCR with AMF-specific primers did not produce any visible products. As such, only the results of the nested PCRs on *Podocarpus cunninghamii* root material are further reported. In addition, the nested PCR results only refer to extraction Methods B (REDExtract-N-Amp™) and C (Puregene), as Method A (Chelex-100™) produced no bands. The results of the primary reactions (Method A) using primers NS1–ITS4 and NS5–ITS4 are shown in Plate 5.3. Primer combination NS1–ITS4 produced visible products of approximately

1,200 base pairs (bp) in seven out of the eight samples, and some samples contained multiple bands. Primary PCR using SSUmAf–LSUmAr yielded no visible bands.

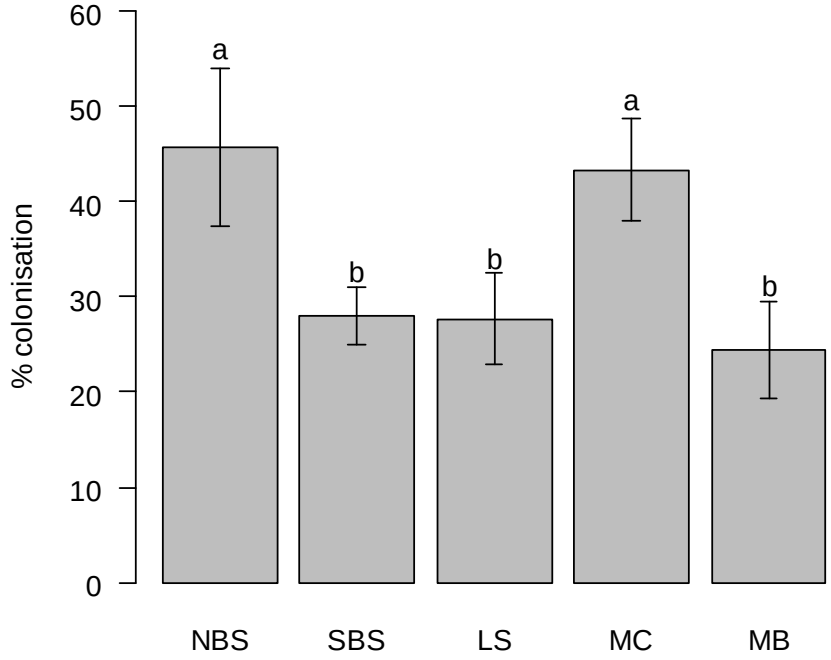

Figure 5.2 – Percent root length colonisation (mean ± 1 s.e.) at the five different sampling sites: Birdwood Station north (NBS) and south (SBS), Longslip Station (LS), Mt Ben Ohau (MB) and Mt Cook National Park (MC). Different letters indicate significant differences.

From the nested PCRs, primers ACAU1660, ARCH1311 and GLOM1310 all produced similar results, with clear bands found across the majority of samples (83%); the products ranged in size from 650 to 850 bp (Plate 5.4). Amplification with primers GIGA5.8R–NS5 and GLOM5.8R–NS5 produced bands of identical molecular weight, with a single band of approximately 500 bp in one of the Mt Ben Ohau samples (Plate 5.5). Primers ARCH1375R–NS3 and GLO1375R–NS3 produced no product at all while primer GLOM1070R–NS3 produced a single band of approximately 550 bp in one of the Mt Cook National Park samples (Plate 5.5). The nested primer combinations of SSUmCf–LSUmBr produced a single band of approximately 1,500 bp, from Mt Cook National Park.

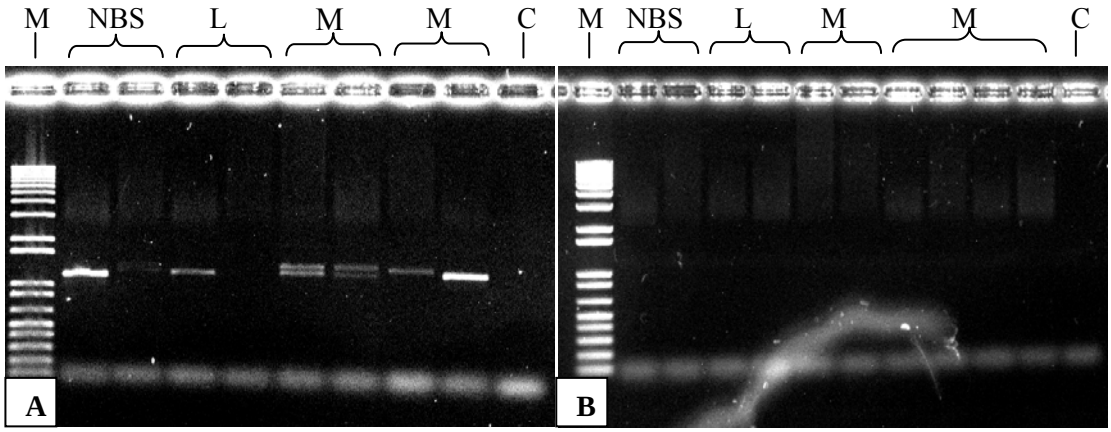

Plate 5.3 – Results of primary PCRs of root samples from Birdwood Station north (NBS), Longslip Station (LS), Mt Ben Ohau (MB) and Mt Cook National Park (MC); C is a negative control; M is 1 kb plus DNA Ladder™ (Invitrogen). A) Products from primers NS5–ITS4; the products are approximately 1,200 bp. B) Outcome from primers NS1–ITS4; no products are visible (a tear in the gel causes the anomalous marking).

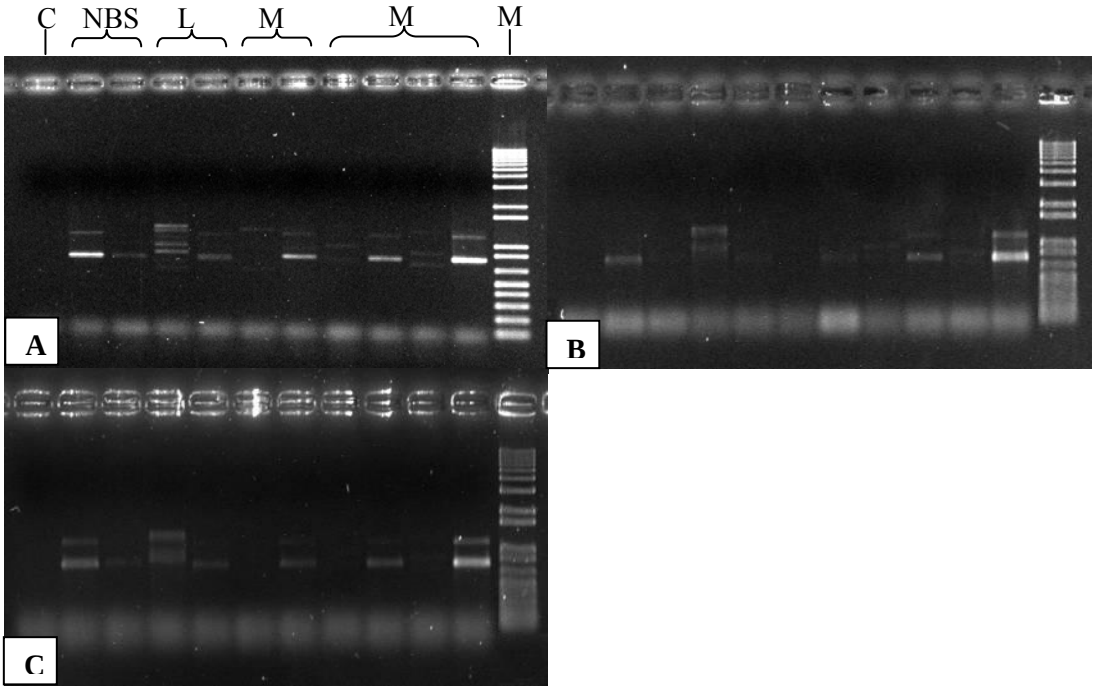

Plate 5.4 – Nested PCR products of root samples from Birdwood Station north (NBS), Longslip Station (LS), Mt Ben Ohau (MB) and Mt Cook National Park (MC). The arrangement of samples and lanes is the same for gels A, B and C. A) ACAU1660 –ITS4; B) ARCH1311–ITS4; C) GLOM1310–ITS4; C is a negative control; M is 1 kb plus DNA Ladder™ (Invitrogen).

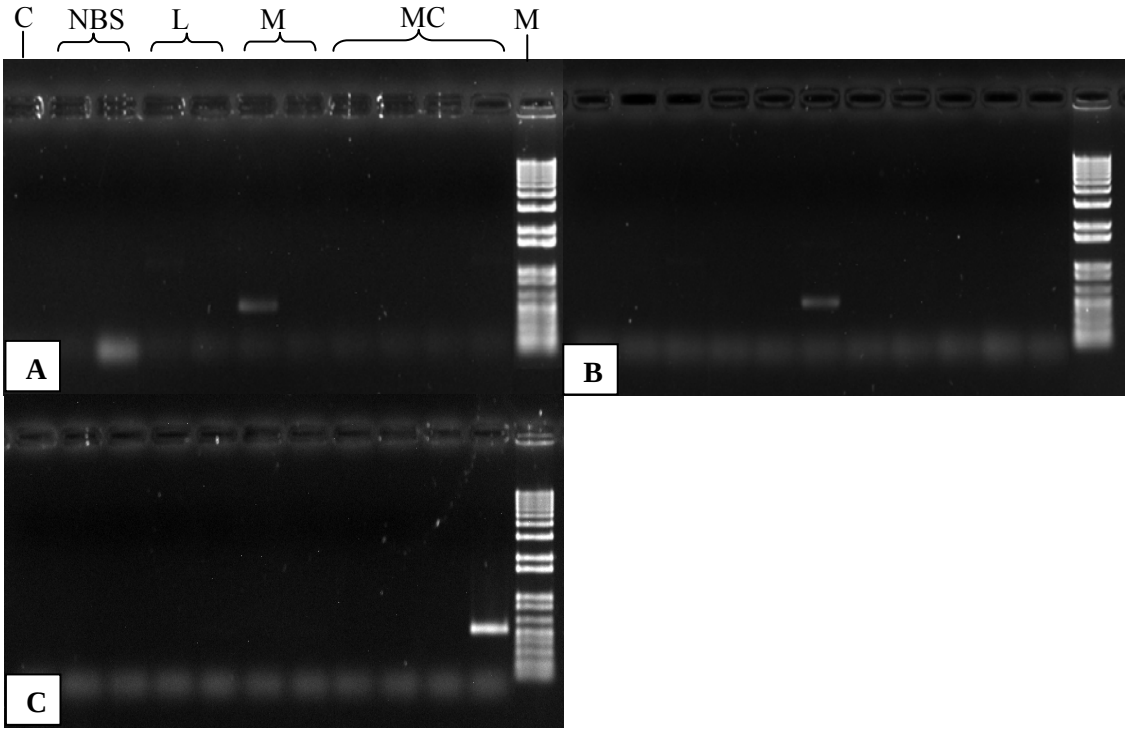

Plate 5.5 – Nested PCR products from Birdwood Station north (NBS), Longslip Station (LS), Mt Ben Ohau (MB) and Mt Cook National Park (MC). The arrangement of samples and lanes is the same for gels A, B and C. A) GLOM5.8R; B) GIGA5.8R; C) GLOM1070R; C is a negative control; M is 1 kb plus DNA Ladder™ (Invitrogen).

The samples that produced clear single bands were selected for DNA sequencing. All bands, except those produced by primers GLOM1070R and SSUmCf, were faint on the gels, indicating weak amplification. The DNA sequencing results for the majority of samples indicated that each of the sequenced bands contained multiple amplimers, which produced overlapping and indecipherable sequence. There were two exceptions to this. The PCR product produced by primers GLOM1070R–NS3 produced a good quality 507 bp sequence (Method B DNA extraction). This was checked on GenBank using the BLAST function, which showed that the sequence had 99% identity to an uncultured Ascomycete fungus (EU350046). Sequencing of the 1,500 bp amplimer produced by primer combination SSUmCf–LSUmBr (Mt Cook National Park, Method C DNA extraction) produced 120 bp of good quality sequence (Appendix 4) and was 95% identical to a single single *Glomus* phylotype (AF452631). This was named Glom-01.

For those PCR products that contained amplimers of different sizes and that were subsequently separated by gel excision and reamplified, DNA sequencing demonstrated that the single bands contained PCR products derived from multiple fungal species (results not shown). Due to the poor quality and overlapping sequence that was produced, it is not possible to say whether the mixed product comprised only AMF species or whether, as in the case of GLOM1070R–NS3, non-target fungal DNA had also been amplified.

5.3.3.2 Single stranded conformation polymorphism (SSCP)

SSCP confirmed that multiple products were present in the nested PCRs (Plate 5.6). An SSCP band for each of the forward and reverse DNA strands was expected. Thus, PCR products appeared to contain up to five individual amplimers (Plate 5.6). Plate 5.6 shows the outcome of one of the gels. The first two methods trialled to extract and amplify DNA directly from excised acrylamide gel slices were unsuccessful. The freeze-squeeze method was successful in yielding minute quantities of DNA and was successfully amplified. The sequences derived from these PCR products appeared to be overlapping sequences of two or more amplimers and was therefore indecipherable.

5.3.3.3 Cloning

A total of 40 colonies were selected at random (10 from each of the four site samples), subjected to PCR and run on 1% agarose gel. A total of seven sequences of good quality were produced from these. Analysis using the BLAST function of GenBank showed that none of the sequences were from AMF. The PCR products generated by primer ACAU1660–ITS4 yielded two Basidiomycete sequences (both *Butlerelfia eustacei*, 90% identical, U85800; both from Mt Cook National Park) and a single Ascomycete sequence (*Alternaria radicina*, 99% identical, AY154704; from Mt Cook National Park). The PCR products generated by primer GLOM1310–ITS4 yielded three Ascomycete sequence (two of *Alternaria radicina*, 98% identical, AY154704; one of an uncultured *Neonectria* clone, 96% identical, FJ552996; all from Mt Cook National Park). The PCR product generated by primer GIGA5.8R–NS5 (from Mt Ben Ohau) did not match any sequence on GenBank.

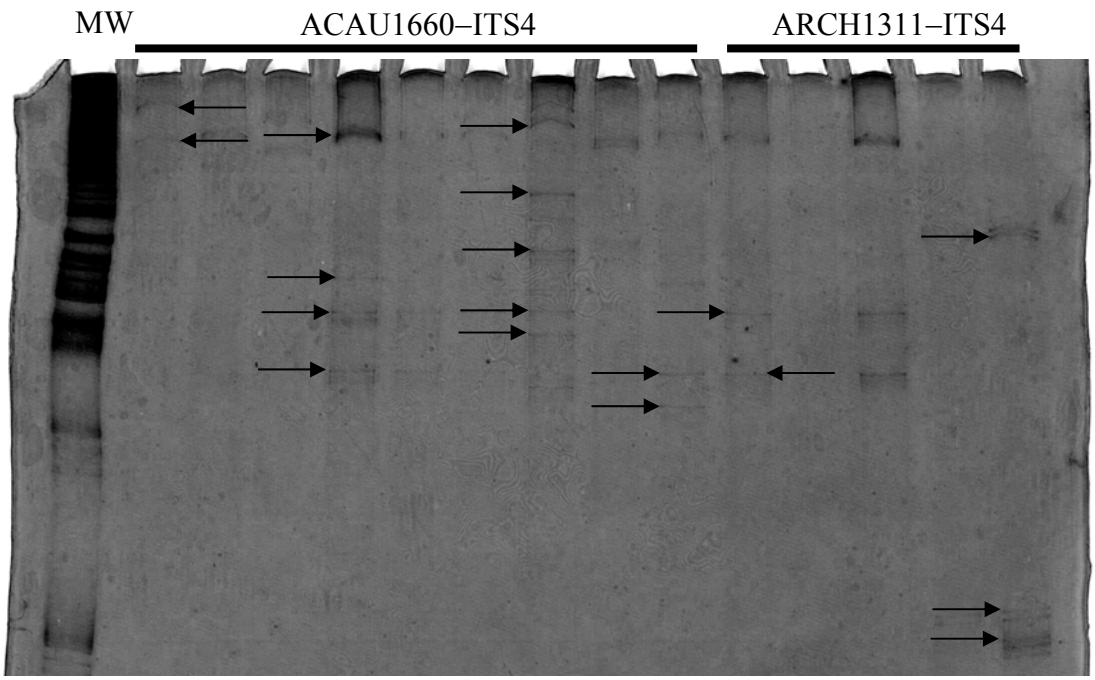

Plate 5.6 – One of the SSCP results of PCR products containing multiple amplimers. MW: Kb plus Mass™ Ladder (Invitrogen). The following nine lanes are positive results from nested PCR using primers ACAU1660–ITS4. The five lanes to the right are positive results from primers ARCH1311–ITS4. Arrows denote bands that were excised and remamplified.

5.3.4 Molecular results with pot cultures

PCR amplification of field samples proved difficult, despite optimising the PCR process, and it was not uncommon for the sequencing reaction to yield non-AMF sequences. In an effort to circumvent this problem, sterile pot cultures of washed *Podocarpus cunninghamii* roots were established to culture the AMF from within the roots. Spores generated by pot culture typically have less contaminating non-target fungi and DNA can be amplified directly from sterilised spores (H.J. Ridgway, pers. comm.). The pot cultures produced spores (approximately 3 spores.g^{-1}) within 4 months (July – November 2008), however these were all immature and the cultures were not harvested for another 2 months (January 2009). At harvest the spore cultures contained approximately 5 spores.g^{-1}. The cultures were left to air-dry then put in cold storage (10ºC) for 4 months (May 2009). At this point several attempts to extract spores from the cultures failed to produce any spores, and closer examination revealed the stored cultures to be heavily infested with mites. The cultures were immediately

wet-sieved to extract all remaining spores. Out of a total of 11 cultures, no spores were found in six, and <5 spores·kg^{-1} were extracted from each of the remaining five. This meant that only a very small number of spores (103) were available for PCR and several of these appeared damaged by mites.

A total of 160 PCRs were carried out on 103 spores. These produced a total of five products of between 650 and 850 bp, which were successfully sequence and queried on GenBank. The sequences were shown to be comprised of two families, Gigasporaceae and Glomeraceae. Of the five sequences, only two were assigned to named species as they fell within a clade of sequences relating to that species: Glom-02 (*Glomus intraradices*; 140 bp) and Glom-04 (*Glomus mosseae*; 137 bp). In total three *Glomus* phylotypes were identified: Glom-02 and Glom-03 (133 bp) came from Longslip Station; Glom-04 came from Birdwood Station. Two *Scutellospora* phylotypes, Scut-01 (228 bp) and Scut-02 (251 bp), were identified from Mt Cook National Park and Longslip Station, respectively. The relationship of all six obtained phylotypes to published New Zealand sequences (Russell *et al.*, 2002) are shown in Figure 5.3. See Appendices 3 and 4 for further sequence information; Appendix 3 provides the BLAST neighbour joining results of each sequence; the electropherograms of each sequence are in Appendix 4.

5.4 Discussion

The analysis of *Podocarpus cunninghamii* root colonisation in combination with the molecular results demonstrated that *Podocarpus cunninghamii* in the high country naturally forms associations with AMF. This is new information and adds to the findings of Russell *et al.* (2002), who found that *Prumnopitys ferruginea, Prumnopitys taxifolia, Dacrycarpus dacridioides* and *Dacrydium cupressinum*, all growing on the west coast, form associations with AMF. The rDNA sequence data collected in this study confirmed that at least two lineages of AMF colonise *Podocarpus cunninghamii*; the Glomeraceae and Gigasporaceae. Two closely related *Scutellospora* sequences were obtained (Scut-01 from Mt Cook National Park; Scut-02 from Longslip Station). Both Scut phylotypes do not belong to any species clade and so cannot be more clearly identified (Appendix 3). The Glom-01 phylotype similarly did not belong to any species clade. Neighbour joining shows that it is

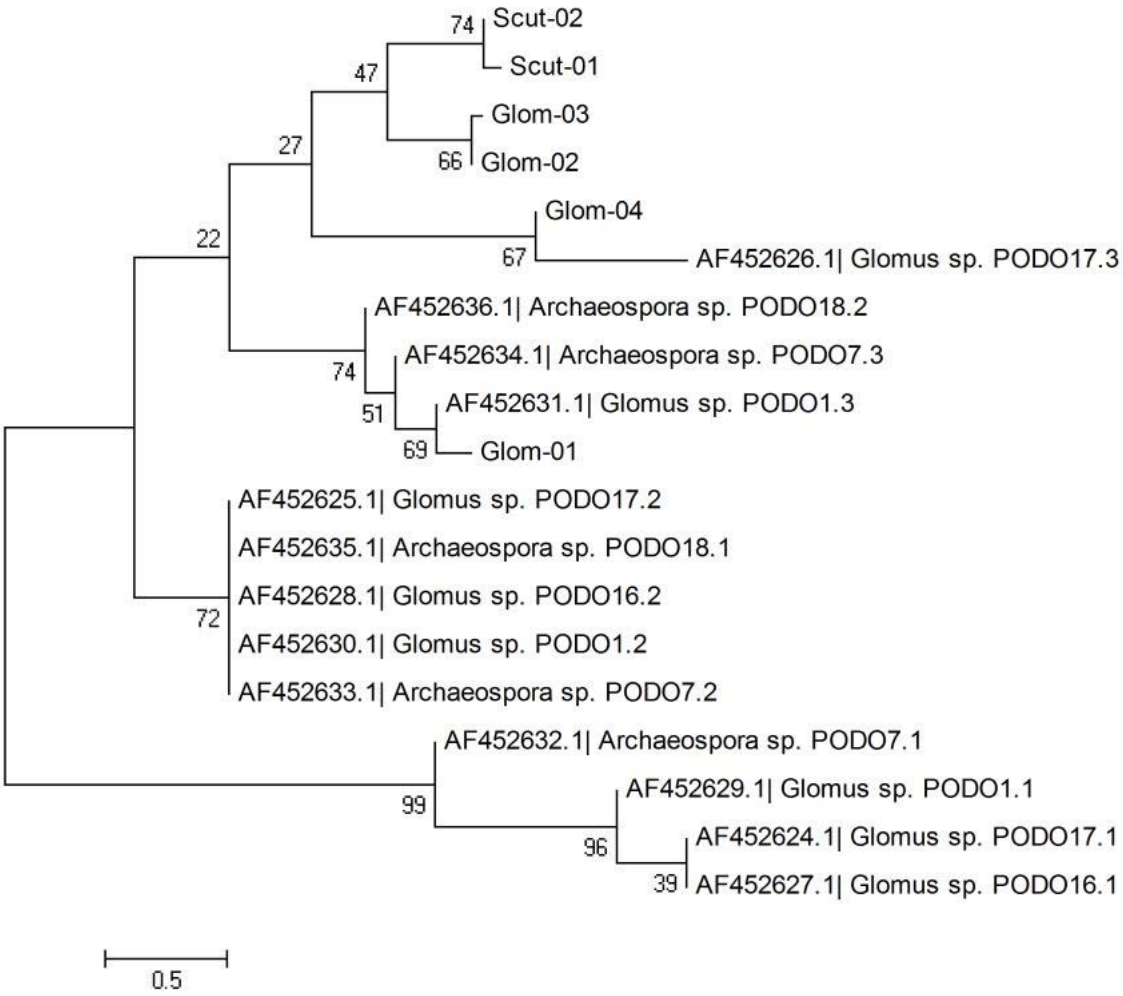

Figure 5.3 – Phylogenetic placement of AMF associated with *Podocarpus cunninghamii* in the eastern South Island high country using previously published sequences of AMF associated with New Zealand podocarps on the west coast (Russell *et al.*, 2002). GenBank accession numbers are given before phylotype names. Numbers on branches are bootstrap values from 1,000 replications.

closely related to a phylotype identified by Russell *et al*. (2002) on the west coast, suggesting shared AMF taxa between *Podocarpus cunninghamii* and other New Zealand podocarps. Glom-04, from Birdwood Station, appears closely related to the *Glomus mosseae* clade (Appendix 3). Within New Zealand, *Glomus mosseae* has been identified in podocarp forest on both the east (Hall, 1977) and west coast (Russell *et al.*, 2002), as well as in a semi-arid region of the high country, suggesting it is a habitat generalist. Glom-02 is closely related to the *Glomus intraradices* clade while Glom-03 appears to find some relation to both the *Glomus intraradices* and *Glomus irregular*e clades (Appendix 3); both phylotypes come from Longslip Station.

It is likely that phylotypes Glom-02 and Glom-03, as well as the two Scut phylotypes, are new records to New Zealand.

The AMF sequences that were obtained were all very short (120-251 bp) compared to those amplified in other studies (Russell *et al*. (2002) obtained sequences of up to 1709 bp). Furthermore, several of the sequences were not very clear, with conflicting peaks for bases present on the electropherograms, especially for sequences Glom-01-03 (Appendix 4). As a result, the confidence with which the sequences can be related to others present on GenBank, beyond a genus level match, is low. The presence of conflicting base readings suggests that more than one amplimer was present in the PCR product and these may represent either multiple closely related AMF isolates or potentially contamination from other non-target fungi. Furthermore, more than one ITS region is known to exist within individual spores and as such individual spores can provide more than one ITS sequence (Sanders *et al.*, 1995). It is also possible that multiple AMF sequences were present within individual AMF hyphae in the roots (Rosendahl, 2008, Rosendahl and Stuckenbrock, 2004).

The overall success of amplifying AMF sequences from *Podocarpus cunninghamii* and grassland root samples was extremely low; attempts to extract and amplify AMF sequences from grassland root samples completely failed. Minimum levels of *Podocarpus cunninghamii* root colonisation were between 20-30%. Therefore, it is possible that some root samples simply harboured no AMF material. Likewise, no confirmation of colonisation of grassland root samples was conducted. However, the possibility of there being no AMF in the grassland root samples seems low, as *Hieracium*, species of which are highly dependent on AMF (Downs and Radford, 2005, Klironomos, 2003, van der Heijden *et al.*, 1998a), was common in each of the sampled grasslands. Compounds, such as polysaccharides, that inhibit PCR may have been present within root material (Rether *et al.*, 1993, Russell *et al.*, 2002). Inhibition of PCR may also have arisen from soil residues on root material, such as humic acid (Tebbe and Vahjen, 1993). Despite this reasoning, it is difficult to reconcile the difficulty in amplifying AMF DNA from both root and spore material given that numerous publications provide evidence for having successfully done so. As a result it has not been possible to compare differences between remnant *Podocarpus cunninghamii* stands and grassland AMF communities.

An important result to arise from this research is that AMF "specific" primers published by Redecker (2000) and Redecker *et al*. (2000) were not specific. The numerous instances where these primers amplified non-AMF DNA from both spore and *Podocarpus cunninghamii* root material are evidence that they amplify DNA from diverse fungal lineages, including the Ascomycota and Basidiomycota. It should be noted that Redecker (2000) and Redecker *et al.* (2000) tested their primers solely on pot culture material, not field material. In additions, the majority of studies identifying AMF by molecular methods are from the northern hemisphere, which may have different soils and different confounding populations of fungi. This being said, caution is advised on the future use of these primers for New Zealand samples. The primers published by Russell *et al.* (2002) were more specific to AMF than the Redecker primers, as they had a lower frequency of amplifying non-AMF DNA. However, they did amplify Ascomycete DNA, and thus cannot be considered as AMF specific. The newer primers published by Krüger *et al*. (2009), appear to have much greater fidelity to AMF, as their use resulted in no instances of non-AMF DNA amplification. A factor in this may be that Krüger *et al*. (2009) tested their primers on field material as well as pot culture material, including material from the southern hemisphere. However, the efficacy of the Krüger *et al*. (2009) primers appears low, as only one sequence was successfully amplified from over 100 amplification attempts (using *Podocarpus cunninghamii* root material). The Krüger *et al*. (2009) primers were not used to amplify spore DNA as the spore work was conducted and concluded prior to their publication.

It is possible that more information on AMF community composition could have been collected if spore morphological identification had been undertaken. However, morphological identification has low accuracy, is extremely time consuming and does not provide an analysis of the AMF community actually colonising plant roots (Clapp *et al.*, 1995, Krüger *et al.*, 2009, Merryweather and Fitter, 1998, Sanders, 2004). Therefore, even if AMF taxa were identified in the soil, based on spore morphology, it would not necessarily follow that those taxa colonise *Podocarpus cunninghamii*. Furthermore, delineation of the AMF community by spore morphology only reflects the diversity of AMF sporulating at that particular time (Clapp *et al.*, 1995). Molecular methods are accurate and can identify intraradical AMF, i.e. AMF actually

colonising the root, and therefore offers a clearer picture of *Podocarpus cunninghamii* AMF community composition compared with spore identification. Although it can be argued that the use of molecular methods can underestimate total AMF diversity as it only measures the diversity of AMF within a particular length of root rather than from a larger soil sample (Hijri *et al.*, 2006, Redecker *et al.*, 2003), it does provide higher accuracy over quantity.

In conclusion, this research confirmed that *Podocarpus cunninghamii* does naturally form associations with AMF in the high country. A total of six sequences were collected belonging to the genera, *Glomus* and *Scutellospora*. Based on sequence identity, four of these species may be new reports for New Zealand, although confidence in some of them is low given their short length and high background on the electropherograms. Several primers published as being AMF specific have been demonstrated as not being so, and caution is advised on their future use in New Zealand.

6 The effect of ex-agricultural and forest AMF on the growth of *Podocarpus cunninghamii* cuttings[1]

6.1 Introduction

Agricultural practices have been shown to change AMF community composition by shifting the community towards dominance by a single family, the Glomeraceae (Helgason *et al.*, 1998, Allen *et al.*, 1998, Oehl *et al.*, 2003). The Acaulosporaceae and Gigasporaceae, which appear to be predominantly forest or woodland AMF, tend to have much lower species richness within agroecosystems (Helgason *et al.*, 1998, Daniell *et al.*, 2001, Hijri *et al.*, 2006). The reasons for this seem related to the intensity of current agricultural practices and land use history. Repeated soil disturbance and increased soil nutrient status can lead to increased selection of Glomeraceae, which have been found to tolerate higher soil nutrient levels but can be less mutualistic (Allen *et al.*, 1998, Egerton-Warburton and Allen, 2000, Johnson, 1993, Maherali and Klironomos, 2007, Oehl *et al.*, 2003).

Abandoned farmland is often subject to ecological restoration, whether by active planting or by promoting natural recolonisation (Cramer *et al.*, 2008). The change in AMF community composition associated with agroecosystems may lead to loss of AMF functional groups necessary for successful restoration of indigenous vegetation. If this is the case, then the change in AMF community composition would represent a biotic threshold potentially constraining or preventing the re-establishment of desired flora (Hobbs and Norton, 2004). It is therefore important to understand whether the shift in AMF community composition within agricultural systems has implications for ecological restoration.

Pre-inoculation of tree seedlings with indigenous forest AMF prior to field planting has been shown to improve seedling growth within abandoned agricultural systems (Allen *et al.*, 2003, Allen *et al.*, 2005). Several other studies have found that AMF

[1] This chapter has been published:
Williams, A., Ridgway, H.J., Norton, D.A. (2011). Growth and competitiveness of the New Zealand tree species *Podocarpus cunninghamii* is reduced by ex-agricultural AMF but enhanced by forest AMF. *Soil Biology and Biochemistry*, **44**: 339-345.

from forest versus agricultural soils have equivocal effects on the growth of forest plants (Asbjornsen and Montagnini, 1994, Gavito *et al.*, 2008, Uibopuu *et al.*, 2009), suggesting that neither inoculum has any benefit, relative to the other, for restoration. However, Fischer *et al.* (1994) found that pasture AMF significantly enhanced growth of a tropical tree compared with forest AMF, suggesting pasture AMF may offer greater benefit.

These studies investigated forest species responses to forest versus agricultural AMF without taking into account plant competition. Exotic pasture species are commonly found on abandoned agricultural sites. These species can represent a major barrier to forest restoration (Cramer *et al.*, 2008). The outcome of plant competition is influenced by AMF identity (Scheublin *et al.*, 2007). As such, investigation into the effects of AMF from forests versus agroecosystems on the success of forest restoration should also investigate how forest plant species respond to competition; in particular, to competition with those plant species that limit restoration success.

The objectives of this study were to 1) identify whether communities of ex-agricultural or indigenous forest AMF had differential effects on the early growth and nutrient acquisition of *Podocarpus cunninghamii*; and 2) quantify whether these AMF communities could alter the competitive relationship of *Podocarpus cunninghamii* and an exotic grass, *Agrostis capillaris*. *Agrostis capillaris* was chosen because it is one of several highly invasive species within the high country and is present in many of the ex-agricultural grasslands where *Podocarpus cunninghamii* restoration could take place.

6.2 Methods

6.2.1 Collection and propagation of cuttings

Podocarpus cunninghamii cuttings were collected from a remnant *Podocarpus cunninghamii* forest on the west face of Mt Ben Ohau, in the Ruataniwha Conservation Park of south Canterbury, New Zealand (44° 10'S, 169° 53'E) during March/April (autumn) 2008 (see Chapter 3 for full site description). Cuttings were selected based on strong apical growth amongst lateral shoots and freedom from visible disease or herbivorous insects. Cuttings of 5-8 cm were clipped from semi-

mature trees, stored within a cool box and transported to glasshouse facilities at the University of Canterbury, Christchurch. Cuttings were stored at 4ºC for up to one week prior to propagation.

The base of each cutting was treated with root promoting compound (Liba 10,000; active ingredient: 10 $g \cdot L^{-1}$ indolebutyric acid) and propagated by insertion into a heated sand bed within an unheated glasshouse. Liquid systemic fungicide was spray applied to the shoots on insertion and once every two weeks thereafter (Yates Greenguard; active ingredients: 102 $g \cdot L^{-1}$ chlorothalonil and 125 $g \cdot L^{-1}$ thiophanate-methyl). A withholding period, where no fungicide was applied for four weeks prior to the planting out of the cuttings, was used in order to minimise any latent fungicide effects on AMF colonisation. A solid fertiliser was dissolved in water (2 $g \cdot L^{-1}$) and applied during the withholding period. In powder form the fertiliser contained the following nutrients: 15% N, 5% P, 30% K, 3% Mg, 0.025% B, 0.01% Cu, 0.07% Fe, 0.04% Mn, 0.004% Mo and 0.025% Zn.

Cuttings were watered six times daily via a misting system that ran for two minutes each time. Temperatures within the rooting medium and the glasshouse were measured using a digital temperature probe and a glasshouse thermometer. Minimum and maximum temperatures within the rooting medium were 7ºC (at 1ºC ambient glasshouse temperature) and 23ºC (at 28ºC ambient glasshouse temperature). Propagation took six months; cuttings were lifted in October (spring) 2008.

6.2.2 Collection and preparation of soils

To replicate field conditions as much as possible the experiment was conducted in a potting mix based on ex-agricultural topsoil collected from the South Island high country. One cubic metre of Pukaki series topsoil, a humose orthic yellow-brown soil (Taylor and Pohlen, 1970), was collected from a formerly grazed grassland on Balmoral Station, west of Lake Tekapo, south Canterbury (44° 02'S, 170° 24'E). Pukaki series soils are well drained and characterised by a weak to moderately structured fine sandy loam to loamy fine sand topsoil (Webb, 1992). The grassland is dominated by the native tussock *Festuca novae-zelandiae*, but has been heavily invaded by *Agrostis capillaris, Anthoxanthum odoratum* and *Hieracium pilosella*;

these invasive species form approximately 75% of current vegetation cover. The site from where the soil was collected had experienced low intensity (<1 stock unit·ha^{-1}) sheep grazing for over 100 years, but was retired from grazing in 1992 (N. Ledgard, pers. comm.; Egerton-Warburton and Allen, 2000). The AMF community was also isolated from this soil and provided the ex-agricultural AMF inoculum (see below).

Forest AMF were collected from a remnant stand of *Podocarpus cunninghamii* forest located within a low intensity sheep grazing system on Longslip Station, west of Omarama, south Canterbury (44° 29' S, 169° 44' E) (see Chapter 3 for full site description). The soil is an upland yellow-grey (palliform) earth (Taylor and Pohlen, 1970). The site on which the remnant occurs has been grazed since the mid-1800s; since the 1950s it has experienced low intensity sheep grazing (<1 stock unit·ha^{-1}) (Williams and Haynes, 1995). Livestock do not appear to access the remnant, as no dung was identified on the forest floor.

Approximately 0.01 m^3 of forest topsoil and 1 m^3 of ex-agricultural topsoil were collected. All soil from each site was collected from the top 20 cm of the soil profile at a single location, once surface vegetation and organic matter was removed. The two soils were kept separate by placing them in large, sealed plastic bags, and transported to the glasshouse facilities in a covered trailer. Both soils were collected in September 2008.

Before the ex-agricultural soil was used as the growing medium for the experiment it was pasteurised in batches in an oven at 120ºC. Each batch, comprising five metal trays, was heated for four hours and turned once after two hours using a trowel that was cleaned with detergent between each use. The temperature and duration of this treatment kills the vast majority of soil organisms, including all pathogenic bacteria and fungi (Hartmann *et al.*, 1997). A total of five 10 g samples of pasteurised soil, each from different batches, were taken and analysed for AMF spores. Spores were isolated by wet-sieving (see below) and analysed microscopically. No live AMF spore material was found.

Once pasteurised the soils were immediately sieved to 2 mm, mixed in equal quantities with sterilised pumice (1-4 mm grade) and stored in sealed plastic bags

before final use; the 50:50 mix of pasteurised soil and sterilised pumice is herein referred to as "potting mix". After pasteurisation soil pH was 5.4, Olsen P was 6.2 $mg \cdot L^{-1}$, total N and total C were 0.2% and 2.1%, respectively, and the C:N ratio was 10.9. For final use the potting mix was transferred to 800 ml square plastic pots placed on benches covered in a 5 cm deep layer of 20 mm diameter washed stones. The stones allowed water to drain freely beneath each pot and minimised inter-pot cross-contamination of AMF.

6.2.3 Inoculation of plants

The experimental design was a randomised complete block with two factors. The first factor had three treatment levels (ex-agricultural AMF vs forest AMF vs non-inoculated control); the second factor had two (+ vs – *Agrostis capillaris*); giving a total of six treatments. All pots received a *Podocarpus cunninghamii* cutting with one of the three AMF treatments; half the pots then received *Agrostis capillaris* seed. Each pot represented one experimental unit, giving six pots per block. There were 60 blocks, giving 360 pots. The position of each pot within each block was randomised, as too was the position of each block within the glasshouse. The position of all pots and blocks was re-randomised once each month to reduce the effect of any microclimatic differences within the glasshouse. The treatments were set up as follows:

Two inoculants were prepared by extracting spores from the forest and ex-agricultural soils separately. Spore concentrations of the field soils were first calculated by wet-sieving five 100 g samples into a 53 μm sieve. Wet-sieving to this pore size is standard practice for surveying natural AMF spore diversity (e.g. Requena *et al.*, 1996, Brundrett *et al.*, 1996). Spores were concentrated by sucrose centrifugation for one minute at 1,000 × *g*. The supernatant was returned to the 53 μm sieve, washed with tap water and transferred to filter paper for spore counts under a dissecting microscope (Brundrett *et al.*, 1996). Once the spore concentrations for each soil were calculated, the required amount of each soil was then wet-sieved into a 53 μm sieve. Sievings were washed into the inoculum medium (the pasteurised soil/sterilised pumice mix) with tap water and thoroughly mixed. The inoculants had a final

concentration of 12.5 spores·g^{-1} of media. The inoculants were stored in a sealed plastic container at 4°C until use.

Successfully rooted cuttings were washed with water to remove sand. A hole was prepared in the potting mix of each pot and the rooting zone of the cutting placed within, plus 20 g of either forest or ex-agricultural AMF inoculum; control cuttings received 20 g of potting mix. A small quantity of potting mix was added above each treatment before pressing down to firmly embed the cutting.

Several studies have suggested that the presence of other soil microorganisms, particularly bacteria, are necessary for efficient arbuscular mycorrhiza formation (e.g. Artursson *et al.*, 2006). Thus, to encourage AMF colonisation 15 ml of an AMF-free microbial wash was added to each of the pots to equalise the microbial community across all pots. This wash, which was prepared by suction filtering 600 g (10% w/v) of forest soil through progressively finer grades to a minimum pore size of 6 µm, is likely to have contained bacteria, yeast and some non-AMF soil-borne fungi (spores and hyphae), but will have excluded larger microorganisms such as AMF, nematodes and protozoa.

After two weeks, 0.1 g of *Agrostis capillaris* seed was added to half the pots. Every pot that received *Agrostis capillaris* also received an additional 20 g of ex-agricultural AMF inoculum. Ex-agricultural AMF was added with *Agrostis capillaris* seed in order to imitate field conditions as much as possible. A 2 cm radius grass-free area was maintained around each cutting to minimise light competition.

Plants were watered twice a day thrice weekly for five minutes via a misting system. A misting system was chosen as it minimises splashing that might have allowed inter-pot AMF cross-contamination. The experiment ran for eight months (October 2008 [spring] – June 2009 [winter]).

6.2.4 Initial measurements

Stem diameter at soil level and above ground shoot length were recorded. Root material was harvested from surplus cuttings, cleared with 10% KOH and stained with

0.05% trypan blue solution (Brundrett *et al.*, 1996). The gridline intersection method (Giovannetti and Mosse, 1980), examining a minimum of 50 line intersections at ×100 magnification, was used to determine if AMF colonisation had occurred while the plants were being propagated.

6.2.5 Harvest and final measurements

Each *Podocarpus cunninghamii* plant was harvested at soil level, and stem diameter and total shoot elongation recorded. Grass shoots were harvested at soil level then placed in an oven for 72 hours at 70 ºC and weighed. *Podocarpus cunninghamii* roots were cleared of soil by washing with water before being placed in an oven for 72 hours at 70ºC then weighed. Equal size fragments of fresh *Podocarpus cunninghamii* and *Agrostis capillaris* roots were collected randomly across all treatments and stored at 4ºC to determine AMF colonisation.

6.2.6 Nutrient analysis

Dried *Podocarpus cunninghamii* roots and *Agrostis capillaris* shoots were ground and 0.1 g placed within a Kjeldahl digest tube. One 3.5 g Kjeldahl digest tablet (Merck) was added per tube with 5 ml conc. H_2SO_4. Samples were left overnight at room temperature to pre-digest. Digestion was then carried out on an AIM500 Block Digestion System (A.I. Scientific) at 200°C for 30 minutes followed by 380°C for 180 minutes. After a cooling period, deionised water was added up to 50 ml and the samples vortexed. Each solution was passed through filter paper then subjected to flow injection analysis (FIA) of total N and total P.

6.2.7 Statistical analysis

Podocarpus cunninghamii relative growth rates were calculated for stem diameter (RGR_D) and shoot length (RGR_L) using the equation: $RGR_x = (\ln(x_f) - \ln(x_i)) / t$. Where x_f and x_i, respectively, are final and initial stem diameter or shoot length, t was the duration of the experiment in months; eight in this case. This provided a monthly relative growth rate, from which an annual rate was extrapolated.

While many *Podocarpus cunninghamii* cuttings produced new growth, many did not. We did not wish to remove the zeros from the data as the numbers of cuttings

producing zero growth appeared related to AMF treatment. As such the growth data was continuous but positively skewed with many zeros. To account for this, relative growth was analysed using a generalised linear model (GLM) with a compound Poisson error distribution (from the Tweedie family of probability distributions) (Jørgensen, 1997), using the statmod and tweedie R packages (Dunn, 2009, Smyth *et al.*, 2009). All significant factors and factor levels were identified through stepwise model simplification with subsequent analysis of deviance; non-significant factor levels were merged to give a pooled mean (Crawley, 2007).

Final *Podocarpus cunninghamii* root biomass (dry weight) was analysed by analysis of covariance, with initial stem diameter as covariate. Final *Agrostis capillaris* shoot biomass was analysed by analysis of variance (ANOVA). Data were log-transformed where necessary to fulfil the assumptions of ANOVA. Significant factors were identified through stepwise model simplification. Significant differences ($p \leq 0.05$) between retained factors were determined *post-hoc* through multiple pairwise *t*-tests using the Benjamini-Yekutieli modification, which controls for the false discovery rate and is considered a more powerful method of conducting multiple comparisons than using the family wise error rate (Benjamini and Yekutieli, 2001).

Percent root length colonised was analysed by ANOVA, with the data square root arcsine square root transformed prior to analysis; control plants were sampled to confirm the absence of AMF colonisation and, because colonisation was absent, were omitted from the analysis. Where significant differences were present, the data was analysed *post-hoc* with Tukey's honest significant differences.

To analyse *Podocarpus cunninghamii* root nutrient concentrations, the 60 blocks were randomly bulked to create five replicates. These were subject to ANOVA, with the data log-transformed where necessary. Significant factors were identified through stepwise model simplification and significant differences determined using Tukey's honest significant differences. All statistical and graphical operations were conducted using R version 2.10.1 (R Development Core Team, 2009).

6.3 Results

6.3.1 AMF colonisation

All *Podocarpus cunninghamii* plants receiving AMF were colonised by AMF (Table 6.1); *Podocarpus cunninghamii* control treatments without *Agrostis capillaris* remained free from infection but those with *Agrostis capillaris* were colonised, due to the ex-agricultural AMF added at the same time as *Agrostis capillaris* seed. Colonisation levels of cuttings inoculated with ex-agricultural AMF were, on average, 7% higher than those inoculated with forest AMF ($F_{2,22} = 3.84$, $p < 0.05$). The presence or absence of grass had no effect on colonisation levels ($F_{2,12} = 0.69$, $p > 0.5$). *Agrostis capillaris* roots were also colonised by AMF; colonisation levels did not differ between treatments ($F_{2,12} = 0.69$, $p > 0.5$).

Table 6.1 – AMF root length colonisation (mean % ± 1 s.e.) of *Podocarpus cunninghamii* and *Agrostis capillaris* for the different grass and AMF treatments. For each plant species, different letters after means indicate significant differences ($p<0.05$).

Grass treatment	**AMF treatment**	***Podocarpus cunninghamii*** (%)	***Agrostis capillaris*** (%)
– *Agrostis capillaris*	Ex-agricultural	30 ± 5^{a}	n.a.
	Forest	19 ± 1^{b}	n.a.
	Control	0 ± 0	n.a.
+ *Agrostis capillaris*	Ex-agricultural	27 ± 3^{a}	27 ± 4^{a}
	Forest	24 ± 1^{b}	21 ± 3^{a}
	Control	21 ± 2^{b}	23 ± 3^{a}

6.3.2 Plant growth

For stem diameter, cuttings inoculated with forest AMF had a growth rate just under twice that of those inoculated with ex-agricultural AMF or control cuttings, but model simplification proved this to be non-significant ($F_{303 \text{ vs } 307} = 1.67$, $p > 0.1$). For shoot growth, cuttings treated with ex-agricultural AMF suffered a significant reduction, with a growth rate that was half that of those treated with either forest AMF or no AMF ($t_{1,307} = 3.05$, $p < 0.01$); the forest AMF and control treatments were statistically equivalent ($F_{304 \text{ vs } 307} = 0.41$, $p > 0.7$). Grass competition was found to have a significant negative effect on *Podocarpus cunninghamii* stem diameter and shoot

growth, with shoot growth being up to three times higher without grass competition (stem diameter: $t_{1,307} = 3.97$, $p < 0.001$; shoot length: $t_{1,307} = 5.65$, $p < 0.001$; Figure 6.1).

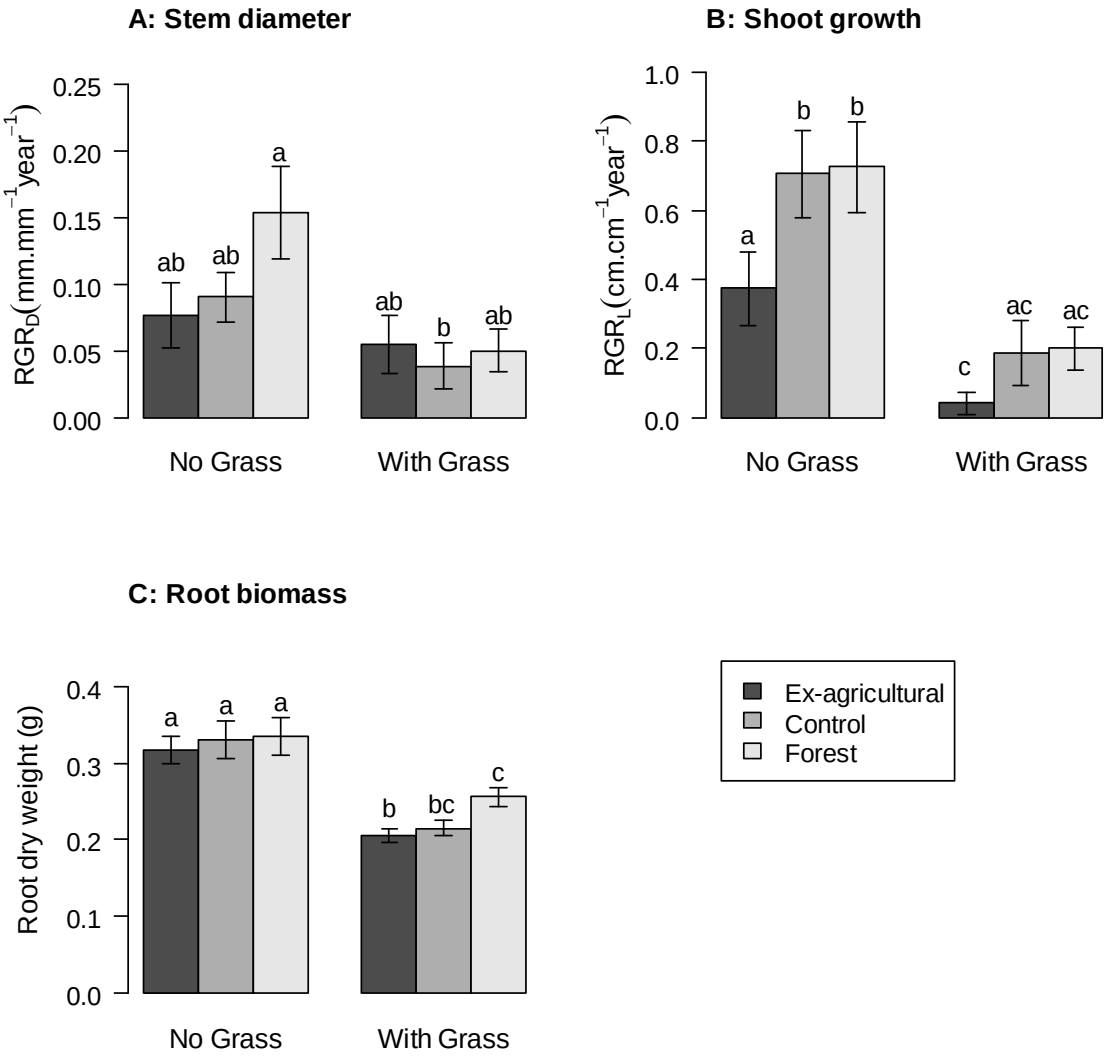

Figure 6.1 – *Podocarpus cunninghamii* growth with AMF and grass treatments. A: relative stem diameter growth; B: relative shoot growth; C: final root dry weight. Different letters indicate significant differences. Bars show 1 s.e. of mean.

For *Podocarpus cunninghamii* root production, ex-agricultural AMF and control treatments did not differ statistically, and their pooled mean was significantly lower than that of the forest AMF ($t_{1,302} = 2.18$, $p < 0.05$), which had approximately 25% more root biomass (0.21 g versus 0.26 g). Initial stem diameter was found to have a significant effect on root production ($t_{1,302} = 6.62$, $p < 0.001$), with larger stems

producing more roots. Competition with *Agrostis capillaris* had a significant negative effect on *Podocarpus cunninghamii* root production ($t_{1,302}$ = 7.86, p < 0.001; Figure 6.1). The final dry weight of roots from cuttings grown without grass was approximately 0.1 g greater compared with those grown with grass. *Agrostis capillaris* shoot production did not differ between any of the treatments (Table 6.2).

Table 6.2 – Final *Agrostis capillaris* shoot biomass and nutrient levels with AMF (mean ± 1 .s.e.). Different letters within variables indicate significant differences.

Variable	Ex-agricultural	Forest	Control
Dry weight (g)	2.31 ± 0.079[a]	2.39 ± 0.079[a]	2.37 ± 0.093[a]
N (%)	1.40 ± 0.053[a]	1.57 ± 0.082[a]	1.45 ± 0.027[a]
P (%)	0.109 ± 0.007[ab]	0.129 ± 0.008[a]	0.103 ± 0.005[b]

6.3.3 Nutrient analysis

Podocarpus cunninghamii root N levels were unaffected by AMF treatment ($F_{2,26}$ = 1.63, p > 0.2; Table 6.3), and there was no grass × AMF interaction ($F_{2,24}$ = 0.36, p > 0.6). Competition with *Agrostis capillaris* almost halved *Podocarpus cunninghamii* root N levels ($F_{1,28}$ = 583.33, p < 0.001). In the absence of *Agrostis capillaris*, *Podocarpus cunninghamii* root concentrations of P were equal with ex-agricultural and forest AMF treatments ($F_{24\ vs\ 26}$ = 2.09, p > 0.1), and were double that of the control treatment ($t_{1,26}$ = 8.45, p < 0.001). Competition with *Agrostis capillaris* had no significant overall effect on *Podocarpus cunninghamii* root P concentrations ($F_{1,26}$ = 2.02, p > 0.1; Table 6.3).

Table 6.3 – *Podocarpus cunninghamii* root nutrient levels (mean ± 1 s.e.) for the different grass and AMF treatments. For each plant species, different letters after means indicate significant differences (p < 0.05).

Grass treatment	AMF treatment	N (%)	P (%)
– *Agrostis capillaris*	Ex-agricultural	1.72 ± 0.047[a]	0.086 ± 0.005[a]
	Forest	1.76 ± 0.064[a]	0.092 ± 0.008[a]
	Control	1.72 ± 0.054[a]	0.045 ± 0.002[b]
+ *Agrostis capillaris*	Ex-agricultural	1.03 ± 0.011[b]	0.067 ± 0.003[c]
	Forest	1.09 ± 0.018[b]	0.080 ± 0.005[a]
	Control	1.06 ± 0.020[b]	0.083 ± 0.004[a]

Agrostis capillaris shoot concentrations of N were unaffected by AMF treatment (Table 6.2). Ex-agricultural AMF and control treatments did not cause any difference in *Agrostis capillaris* shoot levels of P, and their pooled mean was 17% lower than that achieved with forest AMF ($F_{1,13} = 8.19$, $p < 0.05$; Table 6.2).

6.4 Discussion

Ex-agricultural and forest AMF communities had differential effects on the early growth of *Podocarpus cunninghamii* cuttings. Although forest AMF did not result in any significant increase in *Podocarpus cunninghamii* growth compared with controls, ex-agricultural AMF resulted in significantly reduced *Podocarpus cunninghamii* shoot growth. This occurred despite the uptake of P into the roots of *Podocarpus cunninghamii* being unaffected by either AMF community. Furthermore, when in competition with *Agrostis capillaris*, forest AMF caused a significant increase in both *Podocarpus cunninghamii* root production and root concentrations of P compared with ex-agricultural AMF.

The significant reduction in *Podocarpus cunninghamii* growth as a result of inoculation with ex-agricultural AMF may be due to a low C:P exchange between fungi and plant. When nutrients are scarce plants accumulate carbohydrates within root material, but when nutrients are abundant, as in agricultural soils, root carbohydrate allocation diminishes (Hermans *et al.*, 2006). A reduction in root carbohydrate allocation may be detrimental to AMF because they rely entirely upon plant photosynthate for carbon (Smith and Read, 2008). AMF able to survive in a carbon-scarce environment may be more aggressive root colonisers and more effective at acquiring host carbon. As such they would exert a higher plant carbon drain and be less mutualistic (Johnson, 1993). Evidence for this exists; AMF that colonise roots in high N and P soils develop fewer arbuscules, meaning reduced plant-AMF nutrient exchange, and more vesicles, meaning increased storage of nutrients within the fungus (Johnson, 1993, Mosse, 1973). The reason that ex-agricultural AMF did not affect *Agrostis capillaris* shoot production may be because *Agrostis capillaris* has a low mycorrhizal dependency and sensitivity, as defined by van der Heijden (2003), as it showed no AMF treatment effect.

The field from where the ex-agricultural AMF were collected was retired from agriculture in 1992. It would be expected that after 16 years without grazing soil nutrient levels would have reduced, allowing a reversal of any agriculturally driven changes in AMF community composition that may have occurred. Certainly, levels of P in the ex-agricultural soil (6 $mg \cdot L^{-1}$ Olsen P) are as little as one-fifth the amount of three nearby active high country grazing systems (22-30 $mg \cdot L^{-1}$ Olsen P) (A. Williams, unpublished data). Ex-agricultural AMF had a negative effect on *P. cunninghamii* growth, indicating that the AMF community remains dominated by less mutualistic taxa. This suggests that reversing agriculturally driven AMF community changes is more difficult than simply removing grazing for several years. Legacy effects of agricultural practices on AMF community composition have been previously recorded (Hijri *et al.*, 2006). Although in the agricultural field studied by Hijri et al. (2006), phosphate levels remained very high 12 years after a switch from a high fertiliser to low-fertiliser system.

A paucity of forest AMF propagules in the surrounding landscape may be a reason for the less mutualistic nature of the ex-agricultural AMF. A recent study found that similarity in AMF community composition between soil samples decreased as distance between samples increased, suggesting dispersal limitation may affect AMF community composition (Lekberg *et al.*, 2007). The field where the ex-agricultural AMF were collected lies amongst extensive grazing pastures, and the nearest *Podocarpus cunninghamii* forest remnant is 20-30 km away. Wind dispersal of AMF spores has only been recorded for distances up to 2 km (Warner *et al.*, 1987). A major reason why *Podocarpus cunninghamii* forest is not expected to re-colonise the high country without direct human intervention is due to a lack of existing seed sources (Norton, 2006). Such propagule limitation may also apply to AMF.

Soil bacterial communities may be very important for mycorrhizal functioning (Artursson *et al.*, 2006). Urine deposition by sheep is known to have a detrimental effect on the structure of grassland bacterial communities (Rooney *et al.*, 2006), which may in turn have negative effects on the structure and function of mycorrhizal communities (Rooney and Clipson, 2009). The loss of bacterial symbionts in the ex-agricultural field may contribute to the observed reduction in mutualism of the ex-agricultural AMF. The addition of the microbial wash, derived from forest soil, was

intended to overcome any differences in microbial communities between the two soils. However, as it was extracted from forest rather than grassland it may have had a lower efficacy with the ex-agricultural AMF community.

Under conditions of interspecific competition, forest AMF significantly increased *Podocarpus cunninghamii* root production compared with ex-agricultural AMF. This difference showed that the competitiveness of *Podocarpus cunninghamii* within *Agrostis capillaris* grassland could be significantly increased by inoculation with AMF derived from *Podocarpus cunninghamii* forest. The extent of *Podocarpus cunninghamii* root colonisation in the competition control pots was significantly less than for cuttings treated with ex-agricultural AMF as a specific treatment (Table 1). This result showed that reducing ex-agricultural AMF colonisation levels improved *Podocarpus cunninghamii* growth and nutrient acquisition. This further emphasises the negative effect that ex-agricultural AMF had on *Podocarpus cunninghamii* growth. These findings differ to those of Fischer *et al.* (1994), who found agricultural rather than forest AMF caused the greatest increases in tree growth; and to Gavito *et al.* (2008), who found forest and agricultural AMF resulted in equal tree seedling biomass production. These contrasting results highlight the context dependence of the plant-AMF symbiosis, and particularly the influence that plant competition can have on its outcome. The need to take into account plant competition is especially pertinent when considering restoration of ex-agricultural grasslands dominated by invasive species, as is the case here.

In conclusion, the results demonstrated that the inoculation of *Podocarpus cunninghamii* with forest AMF can significantly improve the competitiveness of *Podocarpus cunninghamii* when in competition with *Agrostis capillaris*. Furthermore, in the absence of competition, ex-agricultural AMF caused a significant reduction in *Podocarpus cunninghamii* shoot growth compared with non-inoculated cuttings and those treated with forest AMF. These results strongly suggest that the ex-agricultural AMF community is less mutualistic than the forest AMF community. These results have potential implications for forest restoration, where the pre-inoculation of tree seedlings with forest AMF could improve restoration success. The next step is to investigate the effects of these inocula in the field. Accurate delineation of the ex-agricultural and forest AMF communities through molecular

diagnostics would also help provide a fuller understanding of the functional differences that exist between the communities.

7 The effect of an indigenous and a commercial AMF on the growth of *Podocarpus cunninghamii* cuttings

7.1 Introduction

It is widely recognised that AMF can play a very important role in ecological restoration (Jeffries *et al.* 2003; Jayahandran & Fisher 2008; Bothe *et al.* 2010). The use of indigenous AMF for forest restoration has been demonstrated to improve tree seedling growth, survival and nutrient acquisition in both glasshouse and field environments (Allen *et al.* 2003; Allen *et al.* 2005; Turjaman *et al.* 2008; Urgiles *et al.* 2009). In addition to indigenous AMF, numerous commercially available AMF products are also now available. For restoration practitioners, using such products can be simpler, less time consuming and cheaper than utilising indigenous populations of AMF, which are often uncharacterised and require collection and culturing. However, commercial AMF products typically include easily cultured *Glomus* species that are often exotic to the plants and locations where they are applied (Schwartz *et al.* 2006; Mummey *et al.* 2009). Furthermore, the use of exotic products for restoration can have negative consequences for realising the diversity of AMF found in natural ecosystems (Mummey *et al.* 2009). A further consequence of applying exotic AMF is that it necessitates the introduction of non-native species that have the potential to become invasive (Schwartz *et al.* 2006). Such introductions also have potential aboveground consequences, by improving the fitness of exotic plant species and thus allowing them to become invasive (Wolfe & Klironomos 2005; Desprez-Loustau *et al.* 2007). These concerns are of particular importance for ecological restoration.

Experiments comparing the use of indigenous versus exotic AMF have found that native plants generally show greater growth and nutrient acquisition responses to indigenous AMF rather than exotic AMF (Requena *et al.* 2001; Caravaca *et al.* 2003; Rowe *et al.* 2007). There is also evidence that combinations of local plants with exotic AMF can result in negative plant growth responses more frequently than combinations of locally sourced plants with locally sourced AMF (Klironomos 2003). Interactions between plants and AMF can affect the outcome of interspecific plant competition (Scheublin *et al.* 2007) and have important implications for aboveground

community composition and structure (van der Heijden *et al.* 1998a; Stampe & Daehler 2003; Wardle *et al.* 2004). This is of particular importance to the restoration of abandoned agricultural systems, where exotic plant species can severely inhibit restoration success (Cramer *et al.* 2008).

In this study two AMF species were chosen to investigate the effect of an indigenous AMF versus an exotic commercial AMF on the growth and nutrient acquisition of *Podocarpus cunninghamii*. The two AMF species selected were 1) *Acaulospora laevis* Gerdemann & Trappe (Acaulosporaceae), an indigenous AMF known to colonise *Podocarpus cunninghamii* within New Zealand forests (Johnson 1977), and 2) a *Glomus* based AMF product originally from Australian soil. The study compared the effect that the two AMF species had on *Podocarpus cunninghamii* growth and nutrient acquisition responses when grown individually, to investigate AMF specific effects, and in mixture with *Agrostis capillaris*, to investigate if the different AMF influenced the outcome of competition between the two plant species, the situation that frequently occurs in the field.

7.2 Methods

The collection and propagation of cuttings, collection and preparation of soil for the growth and inoculant media, the initial and final measurements, and statistical analysis were all the same as described in Chapter 6.

7.2.1 Preparation of inoculants

Three inoculants were prepared using the spores of two AMF species. The first was *Acaulospora laevis*, taken from a third generation pot culture initiated from a single spore isolated from New Zealand soil (LU-AL1, Lincoln University Culture Collection, New Zealand). Spores from this culture had been confirmed as *Acaulospora laevis* (HQ148705) by amplification of the ITS1 region of the ribosomoal DNA (rDNA) genes from several spores and querying this sequence against the GenBank database using BLAST (Zhang *et al.* 2000). The other was extracted from a single species AMF product, stated as being *Glomus intraradices* Schenck & Smith (Glomeraceae), and isolated from Australian soil (Myco-Gro®, New Edge Microbials). The identity of spores from this product had been confirmed

by DNA extraction (from approximately 20 spores) and amplification of the large subunit (LSU) of the rDNA using primers FLR3 and FLR4 (Gollotte *et al.* 2004). This sequence (HQ148704) was submitted to GenBank using the BLAST algorithm, where it had 99% similarity to other *Glomus intraradices* isolates (FJ235568, AJ854579, AY842577). However, these isolates have recently been determined to not be *Glomus intraradices*, and actually belong to the *Glomus irregulare* clade (Stockinger *et al.* 2009). In addition, although the commercial AMF product was shown to contain *Glomus irregulare*, it cannot be assumed that that was the only species present. As such, it is herein referred to simply as 'commercial' AMF. All inoculants were prepared to have a final concentration of 10 spores·g^{-1} of media. Spores from both sources were extracted by wet sieving to 53 μm, followed by transferral to the inoculum medium. A third 'mix' inoculum was created by combining equal numbers of extracted spores from the two AMF sources.

7.2.2 Inoculation of plants

Individual pots of single *Podocarpus cunninghamii* cuttings were established, with cuttings receiving one of four AMF treatments (*Acaulospora laevis* vs commercial AMF vs mix inoculum vs non-inoculated control). Plant mixture competition pots were also established where a single *Podocarpus cunninghamii* cutting was placed with 0.1 g of *Agrostis capillaris* seed in addition to one of the four AMF treatments. Therefore there were a total of eight treatments from two factors; factor one: four AMF treatments; factor two: two competition treatments (+ vs – competition with *Agrostis capillaris*). There were a total of 15 replicates, giving 120 experimental units. The position of each pot within each block was randomised, as too was the position of each block within the glasshouse. The position of all pots and blocks was re-randomised once each month to reduce the effect of any microclimatic differences within the glasshouse. The treatments were set up as follows:

Successfully rooted *Podocarpus cunninghamii* cuttings were washed with water to remove sand. A hole was prepared in the pasteurised soil of each experimental pot and the rooting zone of the cutting placed within, plus 20 g of the respective inoculum. A small quantity of pasteurised soil was added above the inoculum before pressing down to firmly embed the cutting. A microbial filtrate was prepared by

suction filtering 800 g (10% w/v) of forest soil through progressively finer grades to a minimum pore size of 6 µm. Each pot received 15 ml to equalise the non-AMF microbial community across all pots. After two weeks, 0.1 g of *Agrostis capillaris* seed was added to the surface of the requisite pots. A 2 cm radius grass-free area was maintained around each cutting to minimise light competition. Plants were watered twice a day thrice weekly for five minutes via a misting system. The experiment ran for eight months (October 2008 [spring] – June 2009 [winter]).

7.2.3 AMF dependency and sensitivity

The dependencies of *Podocarpus cunninghamii*, in monoculture and in mixture, and of *Agrostis capillaris* in mixture, to the mycorrhizal inoculants used (dependency being the difference in plant growth of mycorrhizal versus non-mycorrhizal treatments) were calculated using above-ground biomass values in the following equations (van der Heijden 2003):

$$\text{AMF dependency} = \begin{cases} 1 - (c/\bar{a}), \text{ when } \bar{a} \geq c \\ -1 + (\bar{a}/c), \text{ when } \bar{a} < c \end{cases}$$

where $\bar{a}$ is the average biomass of the inoculated treatments and c is the biomass of the non-AMF control treatment. Mycorrhizal species sensitivity, which is the variation in plant growth response to the different AMF treatments, of *Podocarpus cunninghamii* in monoculture and in mixture, and of *Agrostis capillaris* in mixture, was also determined by calculating the coefficient of variation (the ratio of the standard deviation to the mean) of each plant species' biomass in response to the different AMF treatments (van der Heijden 2003).

7.2.4 Nutrient analysis

Preparation of *Podocarpus cunninghamii* and *Agrostis capillaris* material for flow injection analysis of nutrient content followed that described in Chapter 6. However, in addition to *Podocarpus cunninghamii* root N and P content, analysis of *Podocarpus cunninghamii* foliar N and P content was also undertaken.

7.3 Results

7.3.1 AMF colonisation, dependency and sensitivity

All *Podocarpus cunninghamii* cuttings in the treatments receiving AMF were colonised by AMF (Table 7.1); the control treatments remained free of mycorrhiza. There were no significant differences in colonisation levels between treatments ($F_{1,24}$ = 0.16, $p > 0.6$; $F_{2,24}$ = 2.06, $p > 0.1$; and $F_{2,24}$ = 1.14, $p > 0.3$; for grass treatment, AMF treatment and grass × AMF treatment respectively). *Agrostis capillaris* roots were also colonised by AMF; colonisation levels did not differ between treatments ($F_{2,12}$ = 2.05, $p > 0.1$).

Table 7.1 – AMF root length colonisation (mean % ± 1 s.e.) of *Podocarpus cunninghamii* and *Agrostis capillaris* for the different grass and AMF treatments. There were no significant differences in colonisation levels.

Grass treatment	**AMF treatment**	***Podocarpus cunninghamii*** (%)	***Agrostis capillaris*** (%)
– *Agrostis capillaris*	*Acaulospora laevis*	19 ± 2	n.a.
	Commercial	13 ± 1	n.a.
	Mix	15 ± 3	n.a.
	Control	0 ± 0	n.a.
+ *Agrostis capillaris*	*Acaulospora laevis*	18 ± 2	18 ± 2
	Commercial	17 ± 3	12 ± 2
	Mix	14 ± 2	22 ± 5
	Control	0 ± 0	0 ± 0

Podocarpus cunninghamii and *Agrostis capillaris* had different AMF dependencies, which changed when grown in monoculture or in mixture. *Podocarpus cunninghamii* AMF dependency when in monoculture was 35%, which became negative (-3%) when grown in mixture with *Agrostis capillaris*. *Agrostis capillaris* AMF dependency in mixture was 1%. *Podocarpus cunninghamii* had an AMF species sensitivity of 66% when grown in monoculture, which was reduced to 20% in mixture. *Agrostis capillaris* in mixture had a sensitivity of 21%.

7.3.2 Plant growth

The growth of *Podocarpus cunninghamii* cuttings inoculated with *Acaulospora laevis* and grown independent of *Agrostis capillaris* was 2-4 times greater than in the other treatments. For both stem diameter and shoot length, differences in relative growth rate to commercial, mix and control AMF treatments were not significantly different (stem diameter: $F_{112 \text{ vs } 116} = 1.34$, $p = 0.264$; shoot length: $F_{112 \text{ vs } 116} = 2.30$, $p = 0.063$; Figure 7.1); as such, these treatment levels were combined to give a pooled mean. Cuttings inoculated with *Acaulospora laevis* had a significantly greater relative growth rate compared with the pooled mean treatments (stem diameter: $t_{1,116} = 5.92$, $p < 0.001$; shoot length: $t_{1,116} = 6.38$, $p < 0.001$; Figure 7.1). There was no grass competition × AMF interaction effect on *Podocarpus cunninghamii* stem diameter growth.

The final dry weight of roots from cuttings inoculated with *Acaulospora laevis* and grown independent of grass was between 0.1-0.2 g greater than in the other treatments. Root production did not differ significantly between commercial, mix and control AMF treatments ($F_{104 \text{ vs } 115} = 0.55$, $p > 0.8$; Figure 7.1), and was significantly less than root production of cuttings treated with *Acaulospora laevis* ($t_{1,115} = 4.13$, $p < 0.001$). Root production of cuttings grown with *Agrostis capillaris* was significantly less that those grown without ($t_{1,115} = 6.31$, $p < 0.001$). Initial stem diameter was found to have a significant effect on root production ($t_{1,115} = 5.05$, $p < 0.001$), with larger stems producing more roots. While there was variation in initial stem diameter within each treatment, mean initial stem diameter was even across all treatments, meaning that initial stem diameter did not bias the outcome of any of the treatments. *Agrostis capillaris* shoot production did not differ significantly between any of the AMF treatments ($F_{1,56} = 0.22$, $p > 0.9$; Table 7.2).

Table 7.2 – Final *Agrostis capillaris* shoot biomass and nutrient levels with AMF (mean ± 1 s.e.). There were no significant differences within any of the variables.

Variable	*Acaulospora laevis*	Commercial	Mix	Control
Dry weight (g)	1.91 ± 0.096	2.00 ± 0.127	1.96 ± 0.096	1.93 ± 0.105
N (%)	1.37 ± 0.120	1.29 ± 0.083	1.42 ± 0.131	1.41 ± 0.096
P (%)	0.123 ± 0.021	0.117 ± 0.016	0.119 ± 0.013	0.089 ± 0.013

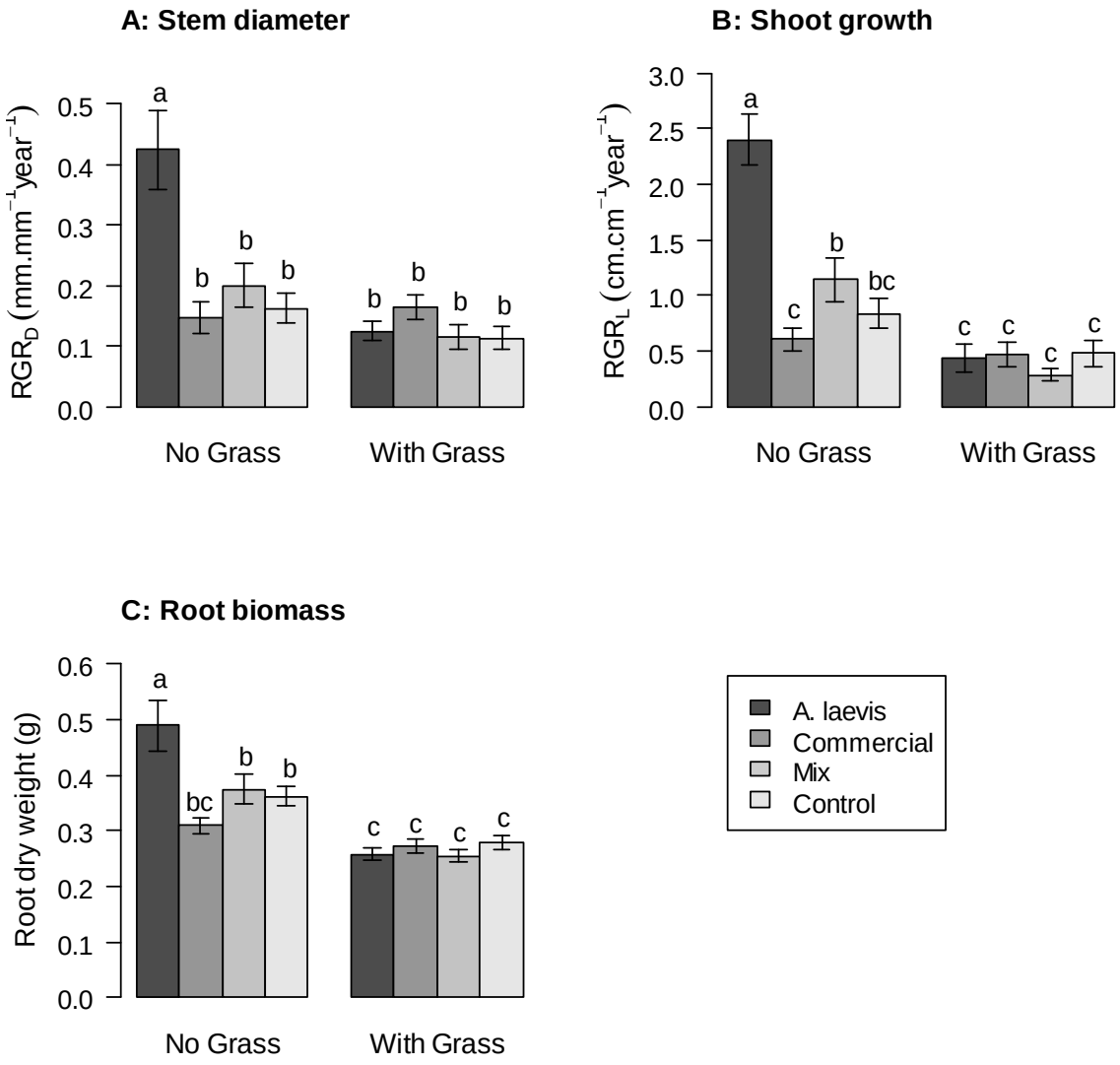

Figure 7.1 – *Podocarpus cunninghamii* growth with AMF and grass treatments. A: relative stem diameter growth; B: relative shoot growth; C: final root dry weight. Different letters indicate significant differences. Bars show 1 s.e. of mean.

7.3.3 Nutrient analysis

Podocarpus cunninghamii foliar and root concentrations of both N and P differed significantly with treatment (Table 7.3). For N, grass competition was found to have a negative effect across all AMF treatments (foliar: $F_{1,33} = 249.86$, $p < 0.001$; root: $F_{1,34} = 398.91$, $p < 0.001$), AMF treatment was highly significant (foliar: $F_{2,33} = 9.24$, $p < 0.001$; root: $F_{2,34} = 8.12$, $p < 0.01$), and there was a strong grass × AMF interaction (foliar: $F_{2,33} = 12.61$, $p < 0.001$; root: $F_{2,34} = 16.92$, $p < 0.001$). In the absence of grass, inoculation with the commercial or mix inoculums led to a

significant reduction in both foliar and root N concentrations. Inoculation with *Acaulospora laevis* resulted in significantly greater foliar N concentrations.

For P, competition had a negative effect on only the *Acaulospora laevis* and mix treatments (foliar: $F_{1,33} = 266.63$, $p < 0.001$; root: $F_{1,34} = 51.86$, $p < 0.001$; Table 7.3). The commercial AMF had no effect on nutrient concentrations, being equivalent to levels in control plants. In the absence of competition, *Podocarpus cunninghamii* cuttings with *Acaulospora laevis* and mix AMF had significantly greater quantities of P than those with the commercial or control treatments (foliar: $F_{2,33} = 89.19$, $p < 0.001$; root: $F_{2,34} = 44.30$, $p < 0.001$). Levels of N and P within the shoots of *Agrostis capillaris* were unaffected by AMF treatment (N: $F_{3,16} = 0.31$, $p > 0.8$; P: $F_{3,16} = 0.91$; $p > 0.4$; Table 7.2).

Table 7.3 – *Podocarpus cunninghamii* nutrient levels (mean ± 1 s.e.). Different letters within source and columns indicate significant differences ($p<0.05$).

Source	***Agrostis capillaris***	**AMF**	**N** (%)	**P** (%)
Foliar	No	*A. laevis*	1.94 ± 0.07^{a}	0.302 ± 0.018^{a}
		Commercial	1.23 ± 0.16^{b}	0.035 ± 0.004^{c}
		Mix	1.76 ± 0.07^{c}	0.189 ± 0.032^{b}
		Control	1.59 ± 0.06^{c}	0.048 ± 0.003^{c}
	Yes	*A. laevis*	1.04 ± 0.04^{d}	0.041 ± 0.000^{c}
		Commercial	1.08 ± 0.04^{d}	0.043 ± 0.002^{c}
		Mix	0.90 ± 0.07^{d}	0.034 ± 0.003^{c}
		Control	0.94 ± 0.04^{d}	0.039 ± 0.002^{c}
Root	No	*A. laevis*	1.94 ± 0.09^{a}	0.254 ± 0.016^{a}
		Commercial	1.41 ± 0.04^{b}	0.059 ± 0.003^{c}
		Mix	1.58 ± 0.04^{b}	0.118 ± 0.017^{b}
		Control	1.75 ± 0.07^{a}	0.066 ± 0.007^{c}
	Yes	*A. laevis*	0.97 ± 0.02^{c}	0.065 ± 0.001^{c}
		Commercial	0.99 ± 0.01^{c}	0.060 ± 0.003^{c}
		Mix	1.11 ± 0.06^{d}	0.071 ± 0.012^{c}
		Control	0.95 ± 0.03^{c}	0.063 ± 0.004^{c}

7.4 Discussion

Podocarpus cunninghamii had a differential early growth response to the different AMF treatments that was dependent on the presence or absence of plant competition. In the absence of competition, *Acaulospora laevis* caused a marked growth response, both above- and below-ground, that was not matched by the other AMF treatments. *Podocarpus cunninghamii* was completely unresponsive to colonisation by the commercial AMF, and in its presence grew as though it was uncolonised.

Competition with *Agrostis capillaris* suppressed the differential response in *Podocarpus cunninghamii* growth (both above- and belowground) to the different AMF treatments. *Agrostis capillaris* had a low mycorrhizal dependency, being unresponsive to any of the AMF treatments, and furthermore, nutrient analysis of *Agrostis capillaris* shoots showed no differences in foliar N and P content with AMF. In light of this, a possible explanation for the lack of response of *Podocarpus cunninghamii* to AMF, when grown with grass, is that *Agrostis capillaris* so aggressively saturated the root space available within each experimental unit that the sheer density of their roots allowed them to deplete the soil of P more effectively than the AMF could acquire it. This outcome would be in accordance with the density dependent model of mycorrhizal effect proposed by Koide & Dickie (2002), and would also explain the dramatic grass-induced reduction in *Podocarpus cunninghamii* mycorrhizal species sensitivity.

In the absence of grass competition, *Podocarpus cunninghamii* acquisition of soil N was AMF dependent. Root concentrations of N for cuttings inoculated with *Acaulospora laevis* were equal to those of control plants; however, foliar concentrations were significantly greater. It was not possible to determine whether the increase in foliar N was due to AMF transfer or rather a natural response to greater root biomass. Irrespective, the result demonstrated that *Acaulospora laevis* either directly or indirectly increased aboveground concentrations of N in *Podocarpus cunninghamii*. Both the commercial and mix inoculums resulted in lower root quantities of N compared with control plants. Interestingly however, foliar concentrations of N in cuttings inoculated with the AMF mix were equal to those of control cuttings while those inoculated with the commercial AMF suffered a

reduction. This suggests that the commercial AMF, which was based on *Glomus irregulare*, may have an antagonistic relationship with *Podocarpus cunninghamii*. It has previously been demonstrated that certain AMF taxa can have detrimental effects on specific host species (e.g. Johnson 1993; Klironomos 2003; Renker *et al.* 2004). While it should not necessarily be assumed that introducing non-indigenous fungi will have a detrimental effect on plant growth (Gazey *et al.* 2004), these results do highlight that commercially available AMF will not necessarily enhance the growth of target plant species.

Johnson (1977) demonstrated that the final biomass of a woody New Zealand shrub was, in general, greatest when inoculated with members of the Acaulosporaceae or the Gigasporaceae, compared with the Glomeraceae. Kubota & Hyakumachi (2004), while not measuring plant responsiveness to different AMF taxa, found that when cucumber, tomato plants and a forest tree species were inoculated with the same soil inoculums, the cucumber and tomato plants had high levels of colonisation predominantly by *Glomus* species while the tree had high levels of colonisation by both *Acaulospora* and *Glomus* species. Further research is needed here, as it is important to identify whether the loss of Acaulosporaceae within agroecosystems (Helgason *et al.* 1998; Daniell *et al.* 2001; Oehl *et al.* 2003) has ecological relevance for the restoration of woody communities.

Future research should also manipulate the intensity of competition by using a range of plant densities. This would elucidate the threshold at which, for a given suite of species, interspecific plant competition and AMF are limiting factors on plant growth. Such work has been conducted with regard to intraspecific competition, and has found that plant responses are dependent on both AMF presence/absence and plant density (e.g. Koide 1991). More recent research has also demonstrated that density dependent plant responses vary across different life forms (Pérez & Urcelay 2009).

In conclusion, this study has found that the early growth of *Podocarpus cunninghamii* was highly responsive to the indigenous AMF, *Acaulospora laevis*, and either unresponsive or negatively responsive to the exotic commercial *Glomus*-based AMF product. This has potential implications for forest restoration, for example by improving the growth and nutrient status of seedlings within the nursery environment,

thereby simultaneously decreasing production times while producing more robust plants. Such pre-inoculation of seedlings with specific AMF prior to field planting may improve the success of restoration plantings (Bothe *et al.* 2010). This research suggests that, for *Podocarpus cunninghamii*, the collection and use of indigenous AMF may be more beneficial for native plant growth than the use of exotic commercial products.

8 A field trial of the effect of the different AMF inoculants on the growth of *Podocarpus cunninghamii* cuttings

8.1 Introduction

Chapters 6 and 7 took a reductionist approach to exploring the effect of AMF on plant growth, nutrient uptake, and interspecific competition in that they explored the effect of different AMF on individual cuttings of *Podocarpus cunninghamii*, and on the interaction between *Podocarpus cunninghamii* and *Agrostis capillaris*, within controlled glasshouse microcosms. The aim of this chapter is to move the research closer towards "ecological relevance" (Read, 2003), and to investigate the potential of AMF for *Podocarpus cunninghamii* restoration in the high country itself. To do this, the effect of five different AMF inoculants (introduced in Chapters 6 and 7) on the growth of *Podocarpus cunninghamii* cuttings was compared in a field trial based in the Mackenzie Basin.

The Mackenzie Basin is a semi-arid intermontane environment receiving approximately 600 mm of annual rainfall. The current vegetation is dominated by native tussock grasses (*Festuca novae-zelandiae* and *Poa colensoi*) heavily invaded by exotic forbs (*Hieracium pilosella* and *Hieracium praealtum*) (Meurk *et al.*, 2002). Prior to human arrival, much of this landscape was dominated by woody vegetation, especially on hill slopes, with *Podocarpus cunninghamii* likely to have been one of the dominant conifer species (see Chapter 2). The Mackenzie Basin is therefore a potential location for *Podocarpus cunninghamii* restoration. As such, conducting a field trial of the effect of AMF on *Podocarpus cunninghamii* growth in the Mackenzie Basin provides the opportunity for proof of concept that is absent from the glasshouse based experiments.

8.2 Methods

8.2.1 Experimental treatments

At the end of the experiments described in Chapters 6 and 7 (June 2009 [winter]), a total of 90 *Podocarpus cunninghamii* cuttings were selected at random out of those treatments that did not include *Agrostis capillaris*, i.e. all the grass-free treatments. This provided a total of six treatments:

- *Acaulospora laevis*
- Commercial AMF
- Mix AMF (50:50 mix of *Acaulospora laevis* and commercial AMF)
- Ex-agricultural AMF
- Forest AMF
- Non-inoculated control

Selected plants were kept in a misted glasshouse (watered twice a day thrice weekly for five minutes) for a further five months before outplanting (November 2009 [spring]).

8.2.2 Experimental setup

Plants were transferred to and planted within an herbivore (lagomorph and sheep/cattle) exclosure plot in the Cass Valley on Glenmore Station, Mackenzie Basin (43° 48’ S, 170° 23’ E) in November 2009. The study site was on a moderately sloping fan (15°, 900 m a.s.l.) in a depleted *Festuca novae-zelandiae* grassland. The experiment was established as a randomised complete block design with one factor: AMF treatment, with a total of five replicates. Plants were spaced 1 m apart in rows of nine, and each row placed 1 m apart. All plants were well watered after planting.

8.3.3 Measurements and statistical analysis

The extent of AMF root colonisation presented in Chapters 6 and 7 provided an indication of the extent of root colonisation of the cuttings in this experiment before they went for outplanting (Table 8.1). Differences in root colonisation between treatments were analysed by one-way ANOVA of arcsine square root transformed proportions. Control plants were omitted from the ANOVA as analysis of root

colonisation confirmed they were AMF-free. Significant differences between treatments were identified *post hoc* using Tukey's honest significant differences.

The growth of *Podocarpus cunninghamii* plants was analysed by analysis of covariance (ANCOVA). Plant height after one growing season was taken as a function of AMF treatment, with initial height as covariate. Stepwise model simplification was used to identify significant differences between treatments. All statistical and graphical operations were conducted using R version 2.10.1 (R Development Core Team, 2009).

8.3 Results

8.3.1 Root colonisation

The extent of root colonisation at the start of the experiment differed with each inoculant (Table 8.1). The most extensive colonisation was achieved by the ex-agricultural AMF, and this was significantly greater than the extent of colonisation achieved by the commercial and mix inoculants ($F_{4,20} = 5.37$, $p < 0.001$). There was no statistical difference in the extent of colonisation between the other treatments.

Table 8.1 – Root colonisation of *Podocarpus cunninghamii* cuttings prior to outplanting (mean ± 1 s.e.). Different letters indicate significant differences ($p<0.05$).

AMF treatment	**Colonisation** (%)
Acaulospora laevis	$19 \pm 2^{a,b}$
Commercial	13 ± 1^{b}
Mix	15 ± 3^{b}
Ex-agricultural	30 ± 5^{a}
Forest	$19 \pm 1^{a,b}$
Control	0 ± 0

8.3.1 Height growth

The growth of cuttings inoculated with *Acaulospora laevis* or forest AMF was up to four times greater than those treated with the commercial or mix inoculums (Figure 8.1). Model simplification showed that the final height of cuttings inoculated with *Acaulospora laevis*, forest AMF and mix AMF were significantly greater than those

inoculated with ex-agricultural AMF, commercial AMF, and non-inoculated controls ($t_{5,22}$ = 2.95, p < 0.01). There was a significant effect of initial plant height ($F_{2,22}$ = 5.23, p < 0.05), with plants treated with forest AMF showing the greatest growth for initial size (Figure 8.1). The final height of plants treated with commercial and mix AMF was less than initial height, due to death of shoots.

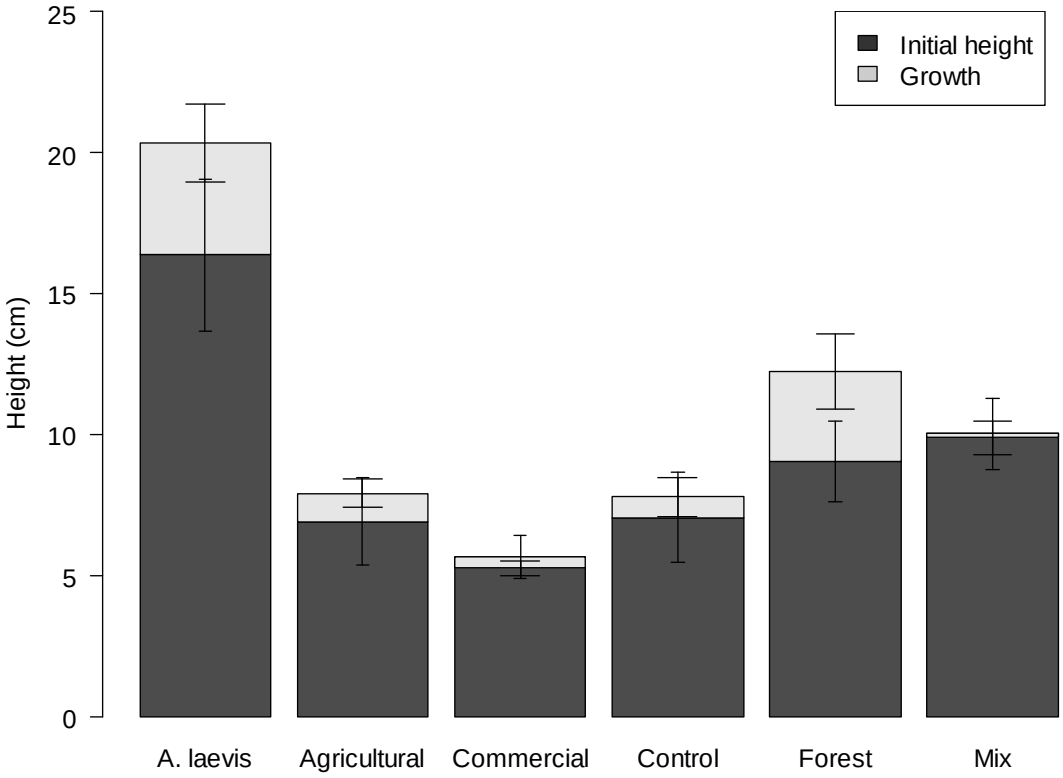

Figure 8.1 – Initial and final heights of *Podocarpus cunninghamii* cuttings with AMF treatment. Plants treated with commercial or mix AMF decreased in height. Bars show 1 s.e. of mean.

8.4 Discussion

After one growing season, the growth response of *Podocarpus cunninghamii* restoration plantings was dependent upon AMF treatment. The indigenous AMF treatments (an indigenous isolate of *Acaulospora laevis* and forest AMF) resulted in significantly greater *Podocarpus cunninghamii* growth in comparison to those cuttings treated with the other inoculants. Inoculation with ex-agricultural AMF did not improve *Podocarpus cunninghamii* growth relative to controls, while the

commercial and mix AMF treatments actually reduced *Podocarpus cunninghamii* height.

The cuttings were treated with inoculants containing different numbers of spores. Plants treated with ex-agricultural and forest AMF (Chapter 6) each received 250 spores, while those treated with *Acaulospora laevis,* commercial and mix AMF (Chapter 7) each received 200 spores. As such, comparisons between plants from Chapters 6 and 7 are not equal and the results should be read with caution. However, the growth differences between treatments are very clear (Figure 8.1), and as such broad trends can be discerned.

Acaulospora laevis has previously been found to colonise a wide range of indigenous New Zealand woody species, including *Podocarpus cunninghamii*, and has been demonstrated to significantly increase biomass production of *Coprosma robusta* compared with numerous *Glomus* isolates (Johnson, 1977). The genus *Acaulospora* is most abundant in woody habitats and can be rare in grasslands and agroecosystems (Helgason *et al.*, 1998, Daniell *et al.*, 2001, Oehl *et al.*, 2003); it is therefore possible that this genus is best adapted to woody hosts. The commercial AMF had a negative effect on *Podocarpus cunninghamii* growth as a result of shoot senescence. This result was also found for plants treated with mix AMF, despite the initial height of these plants being second only to plants treated with *Acaulospora laevis*. This adds to the results in Chapter 7, which suggested that the commercial product, based on *Glomus irregulare* from Australia, has an antagonistic relationship with *Podocarpus cunninghamii*.

Forest AMF resulted in the greatest increase in *Podocarpus cunninghamii* growth for initial plant size. This adds further support to the results of Chapter 6, which showed that the competitiveness off *Podocarpus cunninghamii* within a grassland system was significantly improved by inoculation with forest AMF. The AMF isolated from ex-agricultural soil resulted in no increase in *Podocarpus cunninghamii* growth compared with the non-inoculated control. This is despite the ex-agricultural AMF having the greatest extent of root colonisation of all the treatments at the start of the trial. This indicates that there is no obvious relationship between the extent of root colonisation and plant growth, and suggests that functional differences may exist

between the AMF taxa present within the different AMF treatments used. AMF that aggressively colonise plant roots have been demonstrated to exert a greater carbon drain on host plants (Graham and Abbott, 2000), which may explain the reduction in *Podocarpus cunninghamii* growth with ex-agricultural AMF compared with forest AMF and *Acaulospora laevis*.

Overall, the results of this field trial reinforce the results of Chapters 6 and 7. The differential growth responses caused by the AMF treatments may have important implications for *Podocarpus cunninghamii* restoration. The results suggest that AMF isolated from a *Podocarpus cunninghamii* forest remnant will increase the likelihood of *Podocarpus cunninghamii* restoration success by improving shoot growth. However, there are limitations to the results. The results only present data from one growing season; as such it is not possible to identify whether the growth responses are a direct result of AMF effects in the field or a legacy of nursery treatment. Measurements collected over successive growing seasons would provide this data. In addition, only height measurements were taken, thus root biomass and plant nutrient status are not taken into account. Despite this, the results do highlight the potential benefits to restoration of applying appropriate AMF to *Podocarpus cunninghamii* while within the nursery environment. Those plants treated with *Acaulospora laevis* and forest AMF produced significantly greater growth in the first year after outplanting. This indicates their increased ability to overcome transplant shock and establish once planted in the field. Repeated measurements of these plants over successive seasons will provide further insight.

9 Current nursery practice with regard to mycorrhizae and the propagation of New Zealand's native plants[1]

9.1 Introduction

The majority of New Zealand's plant species develop associations with AMF, including the Podocarpaceae, many forest angiosperms and native grasses (Baylis, 1967, Crush, 1973). *Nothofagus* species form purely ectomycorrhizal (ECM) associations, while *Kunzea ericoides* and *Leptospermum scoparium* are unusual amongst the flora by forming both (Orlovich and Cairney, 2004). The biology of AMF and ECM are very different; for example AMF sporulate within the soil or within plant roots while ECM produce wind-dispersed spores from above-ground fruiting bodies (Smith and Read, 2008). Furthermore, because AM access inorganic rather than organic nutrient sources, their hyphae proliferate within mineral soil; conversely ECM access more organic nutrient sources (Smith and Read, 2008), thus their hyphae proliferate within the litter layer. Mineral soil therefore contains more AM material while leaf litter contains more ECM.

Plants with mycorrhizae can have increased growth rates compared with non-mycorrhizal equivalents, due mostly to increased soil phosphorous uptake (Smith and Read, 2008). Mycorrhizae can also reduce a plant's vulnerability to disease (Whipps, 2004). Application of mycorrhizae to seedlings has been demonstrated to improve establishment and early growth of restoration plants, covering grassland (Richter and Stutz, 2002), shrubland (Requena *et al.*, 2001) and forest (Allen *et al.*, 2005). However, while mycorrhizae can have beneficial plant effects, the association is not always a mutualism. The symbiosis lies on a mutualism-parasitism continuum depending on the specific plant-fungus combination (Johnson *et al.*, 1997); thus, certain mycorrhizae can reduce plant growth.

[1] This chapter has been published:
Williams, A. (2010). Current nursery practice with regard to mycorrhizae and the propagation of New Zealand's native plants. *Ecological Management and Restoration*, **11**: 220-223.

As a consequence of the potential benefits brought by mycorrhizae, there is considerable interest in their use in industrial plant propagation for ecological restoration. Numerous commercial inoculants are available; however these are typically based on easy-to-culture isolates from a particular location within a single country. When sold outside of their native range these products form novel mycorrhizae with the indigenous flora (Mummey *et al.*, 2009, Schwartz *et al.*, 2006). Research has shown that exotic mycorrhizae do not always increase growth of native plants (Requena *et al.*, 2001), and that non-native mycorrhizae are not always able to survive in environments dissimilar to their native habitat (Gianinazzi and Vosátka, 2004). Also, the ecological consequences of introducing non-native mycorrhizae to new habitats is unknown (Schwartz *et al.*, 2006). These are important considerations for ecological restoration. In addition, interest in collecting and using indigenous mycorrhizae for native plant production has been increasing, both within New Zealand (Williams, 2009) and internationally (Corkidi *et al.*, 2008).

In light of the increasing use of mycorrhizae to assist native plant propagation, the differences in biology of AMF and ECM, and the availability of off-the-shelf inoculants, a survey of native plant nurseries with regard to mycorrhizae usage was undertaken. The nurseries surveyed provide a large proportion of their stock for ecological restoration (Davis *et al.*, 2009), thus the survey provides an overview of current nursery mycorrhizae usage for those nurseries with a prominent role in ecological restoration in New Zealand. The purpose of the survey was to ascertain:

1) The extent of mycorrhizae application within native plant nurseries.
2) If native nurseries collect indigenous mycorrhizae or purchase off-the-shelf products.
3) Whether nurseries appreciate the difference between AMF and ECM.
4) If nurseries are maximising efficiency with regard to mycorrhizae application.
5) If fungicide use is common practice.

The responses made it possible to compare nursery mycorrhizae practice with current scientific knowledge, to see, for those nurseries that do invest in mycorrhizae, whether they are investing their resources to greatest effect.

9.2 Methods

Twenty-two native plant nurseries were contacted in New Zealand's North and South Islands. Eighteen were selected from the Davis *et al*. (2009) database (the total number of nurseries in the database), three were found from top Google search hits for the query "New Zealand native plant nursery", and one was identified through a personal contact (D.A. Norton). While the survey does not cover every native plant nursery in New Zealand, it does cover all the major nurseries that together produce over 700,000 plants annually for ecological restoration purposes (N. Ledgard, pers. comm.) and provides almost complete geographical coverage of New Zealand.

Questionnaires were emailed to either the owner or manager of the individual nurseries, with a single reminder email sent to those who did not respond after one month. In every case, the person whom the questionnaire was sent completed the response. These questions were asked verbatim:

1) Are you familiar with the term mycorrhiza and what they do?
2) The use of mycorrhiza:
    a. Are mycorrhiza incorporated into the propagating medium at any stage?
    b. If yes, at what stage? E.g. at initial sowing of seed/insertion of cuttings, or when plants are 'upgraded' to larger containers.
    c. Are commercially available mycorrhiza products used or is material collected from forests?
    d. If material is collected from forests, what is collected? Leaf litter, soil (what depth?)?
    e. How are the mycorrhiza incorporated into the propagating medium? E.g. 50:50 mix with propagating medium.
3) Are fungicides used? If so, at what frequencies and what type (e.g. once a year, foliar applied, systemic action)
4) How would you rate the overall importance of mycorrhiza to plant growth (speed of growth, health of plant), where 0 indicates "of no importance", and 10 indicates "extremely important"?

Within New Zealand, the leaf litter and top surface of the soil (highly decomposed organic matter) is referred to as 'duff'. In the questionnaire, 'soil' refers to the top soil – up to 20 cm depth, including but not limited to duff.

9.3 Results

Eleven questionnaires were returned, giving a response rate of 50%. Five responses were from North Island, six from South Island. Questions were not always answered, or answered in a format not allowing analysis (e.g. a non-numerical answer to question four). This further reduced the sample size for individual questions; sample sizes for each question are provided in Figure 9.1.

Nine of the responding nurseries (82%) actively incorporate mycorrhizae into their propagation setups (Figure 9.1A). Of this nine, one utilises off-the-shelf products exclusively while the majority collect their own (67%) (Figure 9.1B); the remainder use both collected and commercial inoculums (22%). Of those collecting mycorrhizae, the majority collect duff or top soil (33% each) (Figure 9.1C). Of the nine that use mycorrhizae, 56% incorporate inoculum when pricking out (Figure 9.1D), which is when seedlings are lifted from seed trays and individually potted. Of those that responded to question three, 63% use fungicides (Figure 9.1E).

Seven nurseries provided a numeric response to question four, and rated the overall importance of mycorrhizae to plant growth very highly – the average response was 9.1 ± 0.4 (1 s.e.). Of those not giving a numeric response, one felt mycorrhizae were not important within nurseries but carried a value of 8-10 post-planting; another thought they were only important for *Nothofagus*; a third felt more research was required to better understand their importance.

9.4 Discussion

The survey response rate (50%) highlights the fact that the results provide limited coverage of New Zealand's native plant nurseries. This being said, the survey does provide insight into current New Zealand native nursery practice with regard to the use of mycorrhizae. The results show the majority of nurseries surveyed are aware of the value of mycorrhizae and actively attempt to incorporate them into their

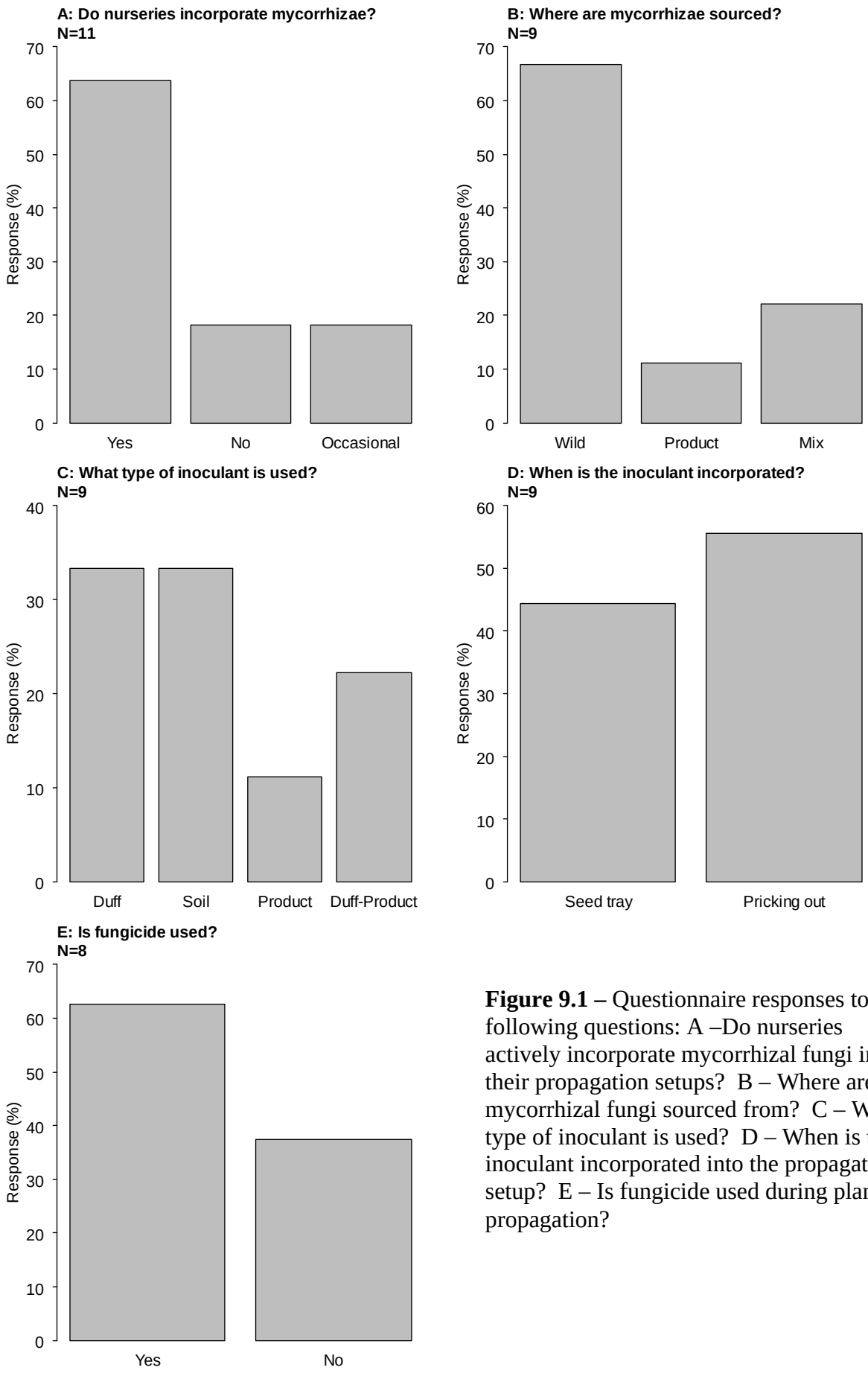

Figure 9.1 – Questionnaire responses to the following questions: A –Do nurseries actively incorporate mycorrhizal fungi into their propagation setups? B – Where are mycorrhizal fungi sourced from? C – What type of inoculant is used? D – When is the inoculant incorporated into the propagation setup? E – Is fungicide used during plant propagation?

propagation setups. In addition, more nurseries expend time and resources collecting indigenous mycorrhizae than use commercial products. This is encouraging, particularly because many of the plants are used in ecological restoration projects. The benefits of non-indigenous mycorrhizae can be limited as well as have unknown ecological consequences (Gianinazzi and Vosátka, 2004, Schwartz *et al.*, 2006). Indigenous mycorrhizae can also dramatically improve the growth of indigenous plants compared with non-mycorrhizal plants or those treated with exotic mycorrhizae (Requena *et al.*, 2001).

However, despite the majority of nurseries using indigenous mycorrhizae, their efforts in collecting them may be being wasted. Two primary sources of inoculum are used: duff or top soil. As described earlier, duff is the leaf litter and highly decomposed organic matter on the soil surface. Given the differences in the biology of AMF and ECM, duff is unlikely to contain quantities of infective AMF material necessary to initiate ecologically representative mycorrhizae. Furthermore with regard to AMF, duff consists mainly of spores rather than hyphae and will therefore only reflect the diversity of AM sporulating at the time of collection; this is not necessarily representative of the true diversity of the AM community (Clapp *et al.*, 1995, Smith and Read, 2008). To collect diverse AM material, the top soil to a depth of 10-20 cm is required. This ensures the rhizosphere, which contains the majority of AM infective material, is adequately sampled (Corkidi *et al.*, 2008).

Notwithstanding the concerns about duff being a poor AMF inoculum, it is important for ECM. ECM access organic as well as inorganic nutrient sources (Smith and Read, 2008), thus their hyphae proliferate in the litter layer. Their spores are also dispersed above-ground. Therefore, unlike AMF, duff is vital to ensure mycorrhizal colonisation of *Nothofagus.*

The results show that current practice invests unnecessary time and effort in attempting mycorrhizal colonisation. The majority of respondents indicated they add inoculum when transferring plants from seed beds or cutting trays to individual containers. To maximise mycorrhizal benefit, inoculum should be incorporated as early as possible, i.e. within seed beds or cutting trays (Gianinazzi and Vosátka, 2004). This maximises plant benefit and reduces both the quantity of mycorrhizal

material and time needed to inoculate a given number of plants. Once inoculated, plants carry their mycorrhizae with them when they are repotted.

The majority of nurseries regularly apply fungicide. This is understandable given the losses that could occur if a root pathogen were to proliferate unchecked. However, the use of soil and systemic fungicides are likely to detrimentally impact mycorrhizae. Research also indicates that appropriate mycorrhizal inoculation can reduce the occurrence of fungal pathogens (Whipps, 2004), meaning mycorrhizae could potentially reduce fungicide usage within nurseries. Research is needed to explore the benefits of fungicide versus early mycorrhizal inoculation for limiting plant losses from root pathogens.

In summary, New Zealand's native plant nurseries appreciate the importance of mycorrhizae for plant propagation and subsequent plant survival after sale. However, the full benefits of utilising indigenous mycorrhizae could be enhanced by a greater understanding of both the associations that different plant species form and the biology of the different types of mycorrhizae.

10 Estimating carbon stocks in stands of *Podocarpus cunninghamii*

10.1 Introduction

As part of the global effort to confront climate change numerous countries became signatories to the Kyoto Protocol, which commits countries to reducing their carbon emissions (UNFCC 1998). The Protocol requires signatory countries to monitor changes in their carbon stocks (gains and losses), including those associated with indigenous forests. As a result there is increasing interest in the quantity of carbon stored within New Zealand's indigenous forests, encompassing both new (restored and regenerating) and old growth forests (Coomes *et al.* 2002; Carswell *et al.* 2008).

Prior to human arrival the eastern South Island high country was forested (McGlone 1989; McGlone 2001; McGlone 2004). Outside of the areas dominated by *Nothofagus*, *Podocarpus cunninghamii* forest was the main forest type (see Chapter 2). Human activity, primarily burning, has converted most of the pre-human cover of *Podocarpus cunninghamii* forest to grassland, however remnant stands still remain. This conversion of forest to grassland would have resulted in massive losses of carbon. On a per hectare basis, living carbon stocks within forests are substantially greater than grasslands (Tate *et al.* 1997), due simply to the difference in biomass of trees and grasses. Consequently, remnant high country stands of *Podocarpus cunninghamii* may represent significant point sinks of carbon in comparison to adjacent induced grasslands; however, little is known of their carbon stocks. Collation of such information is necessary not only for inventories of existing carbon stocks, but also to appreciate the potential of restoration of this forest type to increase carbon stocks.

Estimates of the total amount of carbon stored within New Zealand's different indigenous vegetation types are available. These have tended to be coarse-scale estimates based on broad definitions of vegetation types. For example, based on above- and below- ground estimates of plant biomass, highland podocarp-broadleaved forest has been estimated as containing 274.5 tonnes per hectare ($t \cdot ha^{-1}$) of carbon

(Tate *et al.* 1997). This forest type includes, but is not limited to, upland *Podocarpus cunninghamii-Griselinia littoralis* forests, as defined by Leathwick (2001) (Carswell *et al.* 2008). However, the study by Tate *et al.* (1997) was not based on field biomass measurements. Instead, they used the Vegetation Cover Map of New Zealand (Newsome 1987) in combination with National Vegetation Survey (NVS) data and available literature to determine and interpolate biomass and carbon values for indigenous forest types. More recently, carbon stocks within South Island forest and shrublands have been estimated using field measurements (Coomes *et al.* 2002). They followed a transect starting within intact forest on the west coast and finishing in the human induced semi-arid shrublands east of the Main Divide. Their estimates of combined above- and below-ground living carbon stocks were 174 $t \cdot ha^{-1}$ in forest and 26.7 $t \cdot ha^{-1}$ in shrubland. The carbon stock estimates of Coomes *et al.* (2002) are almost certainly at least partly derived from forest and shrubland associations containing *Podocarpus cunninghamii*. However, they do not provide information on the different forest or shrubland classes included in their sampling. The only figure available relating specifically to *Podocarpus cunninghamii* comes from *Podocarpus cunninghamii-Nothofagus solandri-Phyllocladus alpinus* forest at Twizel, where live carbon stocks were estimated at 160 $t \cdot ha^{-1}$ (Kirschbaum *et al.* 2009), based on forest biomass models (Hall & Hollinger 2000). To gain a more complete picture of carbon stocks within high country stands of *Podocarpus cunninghamii*, information from more than one site is required.

In the last few decades increasing areas of grazing grassland have been retired and placed within the New Zealand government's public conservation estate (DOC 2010). As a result there are now extensive grassland areas with the potential to restore pre-human forest flora (Walker et al., 2009). In addition to the Crown Estate, private land also has the potential for indigenous restoration through the afforestation of unproductive and erosion-prone marginal lands (Kirschbaum *et al.* 2009). Although indigenous tree species have lower carbon sequestration rates than exotic species, indigenous afforestation of grasslands could contribute significantly to the government's Kyoto carbon reduction commitments (Kirschbaum *et al.* 2009). Such restoration would have additional benefits in terms of soil erosion control, enhancement of indigenous biodiversity, as well as provide a potential income source to landowners through the Permanent Forest Sink Initiative (MAF 2007). In light of

the evidence for the composition of the pre-human high country forest flora, indigenous forest restoration in the high country is likely to incorporate *Podocarpus cunninghamii*. Estimating the carbon sequestration rate of *Podocarpus cunninghamii* is therefore necessary to estimate the contribution that its restoration could make to carbon storage within these environments.

The aim of this research was to estimate the carbon stocks held within the living biomass of *Podocarpus cunninghamii* within several high country remnants, based on field measurements to estimate standing biomass. Estimates of carbon stocks are presented for *Podocarpus cunninghamii* stands along a west-east high country rainfall gradient. In addition, mensurational data were combined with tree ring counts to model *Podocarpus cunninghamii* diameter increment over a 100 year period. From this was derived a first estimate of *Podocarpus cunninghamii* carbon sequestration rates in the high country. This data is then considered within the context of ecological restoration and New Zealand's Kyoto commitments.

10.2 Methods

10.2.1 Data collection

All of the data used in this study is based on the population dynamics dataset of Chapter 3 (section 3.2.2.2). As such all the data comes from six sites: Birdwood Station, Mt Ben Ohau, Mt Dobson, Mt Cook National Park, the Upper Lawrence and the Upper Hapuku (see Chapter 3 for site descriptions); a summary of each stand is given in Table 10.1.

Table 10.1 – Stand characteristics of *Podocarpus cunninghamii* at the six study sites.

Site	**Altitude** (m)	**Plot size** (ha)	**Stem density** (stems·ha^{-1})	**Basal area** (m^2·ha^{-1})	**Average height** (m)
UH	850	0.2	250	35.29	9.90
UL	850	0.2	250	44.65	12.25
MC	800	0.4	125	6.17	6.97
MD	850	0.21	238	14.13	6.05
MB	750	0.12	416	10.02	4.04
BS	700	0.05	1000	6.27	3.68

10.2.2 Estimation of living carbon stocks

The procedure to estimate carbon stocks followed that of Coomes *et al.* (2002), Kirschbaum *et al*. (2009) and Carswell *et al.* (2009), all of whom estimated carbon stocks of New Zealand forest flora. Firstly, aboveground tree biomass (AGB) was estimated by using dbh and height data in the following equation:

$$AGB = 0.0000598 \times \rho \times (d^2h)^{0.946} \times (1 - 0.0019d) + 0.03d^{2.33} + 0.0406d^{1.53} \quad \text{(Eqn 10.1)}$$

Where d is dbh (cm) and h is tree height (m); ρ is wood density ($kg \cdot m^{-3}$). No published data is available for *Podocarpus cunninghamii* wood density. As such, the average wood density derived for the closely related *Podocarpus totara* in Northland (446 $kg \cdot m^{-3}$) was used instead (Bergin *et al.* 2008)[1]. The root biomass of each tree was assumed to be 25% of aboveground biomass, and together gave total tree biomass. Total tree carbon stock was then assumed to be 50% of total tree biomass. Individual tree carbon stocks were then summed for each plot, giving a plot carbon stock. Plot carbon stocks were then multiplied by 1/plot size (Table 10.1), to give a tonnes per hectare ($t \cdot ha^{-1}$) carbon stock for each site. The differences in carbon stocks with LENZ derived annual soil moisture deficits were analysed by linear regression.

10.2.3 Estimation of carbon sequestration rate

Counts of tree rings were undertaken on all cores that reached the pith, allowing as complete a chronology of rings as possible to be analysed. The width (mm) of every block of 10 rings was measured, providing a rate of radius increment for each 10 year period of each tree's life to the present day. This increment was doubled to provide a diameter figure. The mean growth rate for each 10 year period was then calculated for all cores. All data over 120 year's age was excluded due to the paucity of samples. Mean diameter annual increment (MAI) was then modelled using the following equation (where a is age in years):

$$MAI = 0.000000655a^3 - 0.000237a^2 + 0.0173a + 1.16 \quad \text{(Eqn 10.2)}$$

[1] Given the much slower growth rate of *Podocarpus cunninghamii* compared with *Podocarpus totara*, using Northland *Podocarpus totara* wood density is likely to underestimate high country *Podocarpus cunninghamii* wood density.

Cumulative MAI was then used to estimate *Podocarpus cunninghamii* diameter increment with age. The relationship between *Podocarpus cunninghamii* dbh and height was modelled using a log-log linear regression. This relationship was then used to predict *Podocarpus cunninghamii* height at the respective diameters in the cumulative MAI model. These predicted diameters and heights were then used in the process described in 10.2.2, along with two example stocking densities (250 stems·ha^{-1} and 1000 stems·ha^{-1}), to estimate per hectare carbon stocks over a 100 year period. Both per hectare carbon stocks were divided by the average cosine of the slopes of all sites. All modelling, statistical analysis and graphing were done using R version 2.10.1 (R Development Core Team 2009).

10.3 Results

10.3.1 Estimation of living carbon stocks

A total of 50 trees were sampled at each of the six study sites, giving a total of 300 sampled trees. Per hectare estimates of above- and below-ground carbon within *Podocarpus cunninghamii* at the six study sites are shown in Table 10.2. Per hectare carbon stocks are highest at sites with the greatest basal area. Thus, the greatest unit quantities of carbon are stored at Upper Lawrence and Upper Hapuku, while the lowest are at the driest site, Birdwood Station. The overall mean carbon stock across all sites was 51.9 ± 6.5 t·ha^{-1} (± 1 s.e.).

Table 10.2 – Carbon stocks of *Podocarpus cunninghamii* at the six study sites.

Site	**Altitude** (m)	**Stem density** (stems·ha^{-1})	**Soil moisture deficit** (mm·yr^{-1})	**Min. temp.** (ºC)	**Per hectare C stock** (t·ha^{-1})
UH	850	250	0.00	-1.53	100.5
UL	850	250	0.00	-1.95	130.1
MC	800	125	0.00	-2.30	17.3
MD	850	238	0.00	-2.30	38.0
MB	750	416	30.13	-2.45	18.2
BS	700	1000	98.73	-3.07	7.3

The data from Mt Cook National Park had a strong skewing effect on the data, as it has no annual soil moisture deficit and a small carbon stock. Its removal from the

model drastically improved the trend, showing a significant negative relationship of decreasing carbon stocks with increasing annual soil moisture deficit ($t_{1,3}$ = -3.21, p = 0.049) (Figure 10.1).

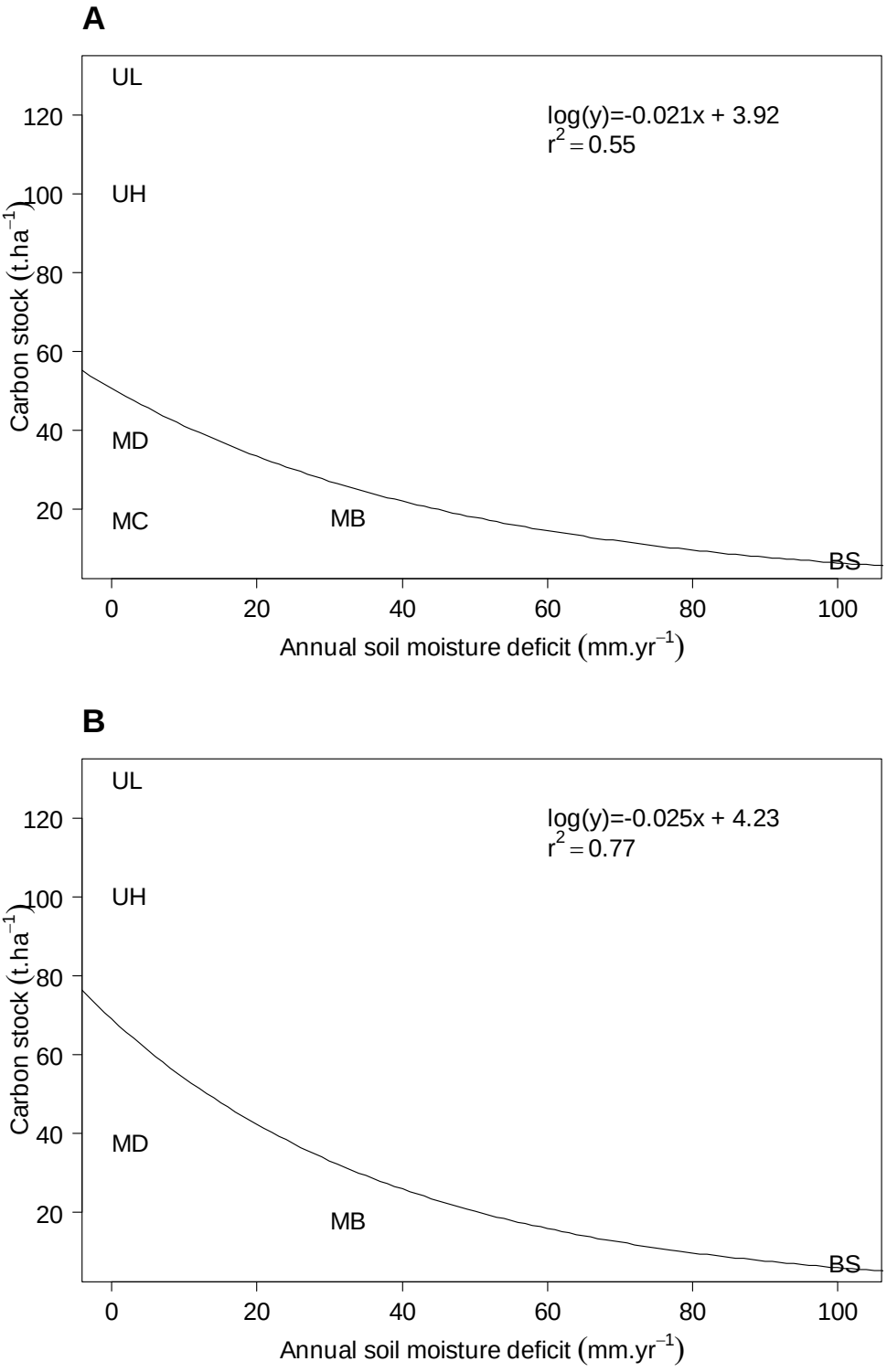

Figure 10.1 – Relationship between carbon stocks and annual soil moisture deficit. A: All six sites; B: Excluding Mt Cook National Park.

10.3.2 Estimation of carbon sequestration rate

A total of 47 trees were used to model MAI (Figure 10.2A). The data was heavily biased towards trees aged 10-50 years (mean n per 10 year age class = 44), with less data available for trees 60-100 years (mean n per 10 year age class = 16); only a few trees provided data for 110-160 years (mean n per 10 year age class = 2). In addition, the data is heavily biased towards sites with colder and drier growing conditions; of the 47 trees sampled, 25 come from the dryland sites Birdwood Station and Mt Ben Ohau; 11 come from Mt Cook National Park, which like Birdwood Station and Mt Ben Ohau has significantly lower winter minimum temperatures compared with Upper Lawrence and Upper Hapuku (Table 10.2, Chapter 3). MAI shows a trend of increasing diameter increment to a maximum of 1.5 mm after 50 years, before steadily declining to 1.2 mm at 100 years. Cumulative MAI is shown in Figure 10.2B; the expected dbh of a tree after 100 years is 14.3 ± 1.4 cm (± 95% CI). The relationship between dbh and height, based on the full dataset (n=300), is shown in Figure 10.3. The predicted height of *Podocarpus cunninghamii* after 100 years is 5.2 ± 0.2 m (± 95% CI) (based on dbh of 14.3 cm).

The estimated carbon stock of a single *Podocarpus cunninghamii* tree after 100 years (14.3 cm dbh and 5.2 m height) is 20.2 ± 4.2 $kg \cdot m^{-3}$ (± 95% CI). The per hectare 100 year carbon stocks of stands of trees at 250 and 1,000 $stems \cdot ha^{-1}$, as well as their annual carbon sequestration rates, are given in Table 10.3. The annual sequestration rate for both stocking densities is low, and is four times greater for 1,000 $stems \cdot ha^{-1}$ compared with 250 $stems \cdot ha^{-1}$.

Table 10.3 – Per hectare carbon stocks of 100 year old hypothetical stands of *Podocarpus cunninghamii* at two different stocking densities, and their annual sequestration rate (± 95% CI).

Stocking density ($stems \cdot ha^{-1}$)	**Annual sequestration rate** ($t \cdot ha^{-1} \cdot yr^{-1}$)	**Per hectare C stock** ($t \cdot ha^{-1}$ after 100 years)
250	0.1 ± 0.02	9.0 ± 1.9
1,000	0.4 ± 0.08	36.0 ± 7.6

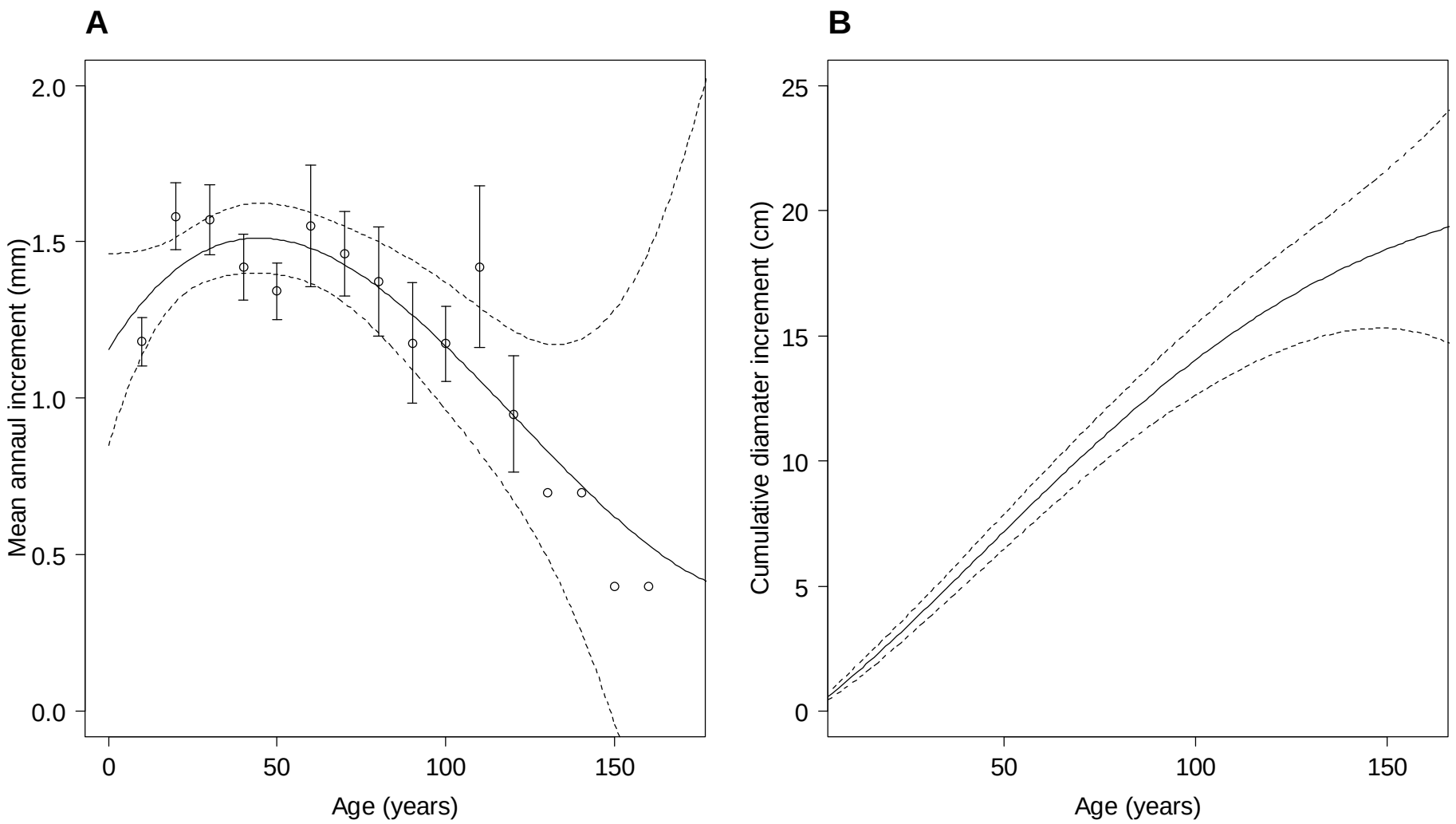

Figure 10.2 – A: Mean annual increment of high country *Podocarpus cunninghamii* (solid line). The points show mean annual increment for each 10 year age class ± 1 s.e. No error bars are shown over 120 years as points are based on single trees; B: Cumulative MAI. The dotted lines on both Figures show the 95% confidence interval.

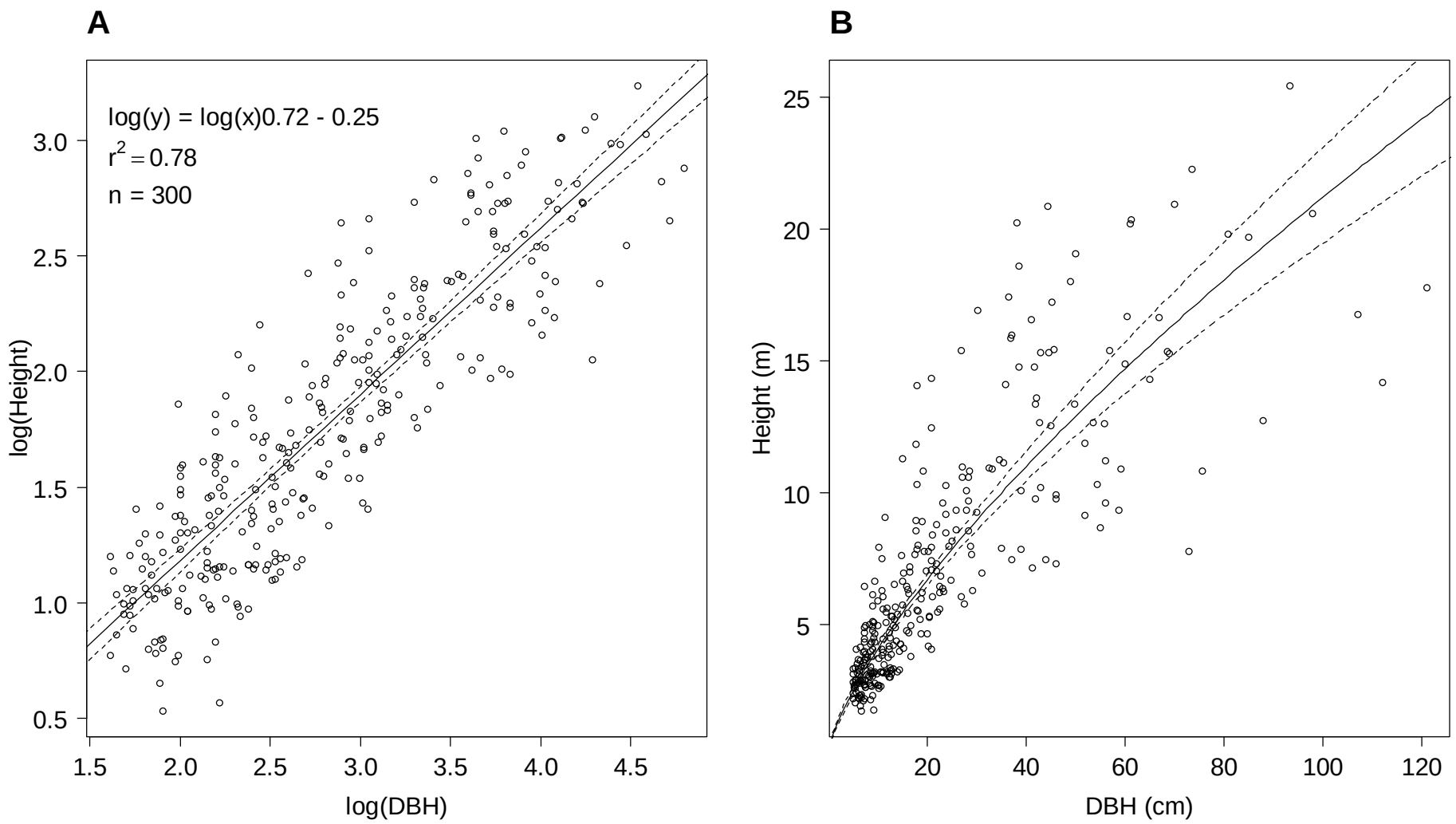

Figure 10.3 – A: log-log relationship of dbh and height; B: unlogged relationship of dbh and height. The dotted lines on both Figures show the 95% confidence interval.

10.4 Discussion

10.4.1 Estimation of living carbon stocks

The results here provide a first estimate of live above- and below-ground carbon stocks within high country stands of *Podocarpus cunninghamii*. The mean carbon stock value of 51.9 ± 6.5 $t \cdot ha^{-1}$ (± 1 s.e.) is much lower than those previously published for New Zealand's indigenous forests; Tate *et al.* (1997) estimated 274.5 $t \cdot ha^{-1}$ and Coomes *et al*. (2002) estimated 174 $t \cdot ha^{-1}$. However, Tate *et al.* (1997) assigned their figure to highland podocarp-broadleaf forest arbitrarily, while the Coomes *et al.* (2002) study included biomass values of all tree species within plots and also sampled west coast stands with much greater biomass. The present study only measured the biomass of *Podocarpus cunninghamii*. It is also somewhat unfair to compare the carbon stocks of intact west coast forest against the low biomass stands of *Podocarpus cunninghamii* in the eastern drylands. The high country climate varies widely; areas close to the Main Divide receive high levels of annual rainfall (>2,000 mm) while further east annual rainfall declines to <600 mm. The eastern drylands, which were particularly susceptible to historical fire-driven forest loss (McGlone 1989), have been heavily deforested and surviving stands are highly degraded. In contrast, areas close the Main Divide still support extensive stands of relatively intact *Podocarpus cunninghamii* forest (Wardle 1991). Stocks of carbon are lower within degraded versus intact forest (Gibbs *et al.* 2007), and as such per hectare stocks of carbon within remnants closer to the Main Divide would, in general, be expected to be greater than stocks further east.

It is likely that the figures given for the relatively intact stands of *Podocarpus cunninghamii* in the wetter parts of the high country are likely to more closely resemble published figures of indigenous forest carbon stocks. On the other hand, the degraded stands in the drylands are likely to more closely resemble published figures of indigenous shrubland carbon stocks. This certainly appears to be the case; Upper Lawrence and Upper Hapuku, which are both closed canopy stands, have estimated carbon stocks of 130.1 and 100.5 $t \cdot ha^{-1}$, respectively. *Podocarpus cunninghamii* is by far the largest species in these forests, and the majority of standing biomass is probably held within them. As such, these estimates can be seen as conservative minimum estimates of live carbon within these wet and relatively mild forests. In

addition, these estimates ignore soil carbon pools, which can represent a substantial amount of additional carbon, although less than the standing pool (Carswell *et al.* 2008; Kirschbaum *et al.* 2009). With regard to the dryland sites, the estimated carbon stocks of Mt Ben Ohau and Birdwood Station are 18.2 and 7.3 $t \cdot ha^{-1}$, respectively, which are similar to the 26.7 $t \cdot ha^{-1}$ published for indigenous eastern shrublands, of which 5.9 $t \cdot ha^{-1}$ was contributed by trees (Coomes *et al.* 2002). Again, the figures derived in the present study only include the contribution of *Podocarpus cunninghamii*, thus ignoring the actual shrubland component; although it is considered that the shrubland component would be relatively minor.

Despite the estimates of carbon stocks in the dryland stands appearing low relative to the wetter stands, they are still far greater than carbon stocks estimated for improved and unimproved pasture (2.1–2.9 $t \cdot ha^{-1}$ (Tate *et al.* 1997)). The figure for Mt Ben Ohau carbon stocks, which has a fractured canopy (c. 46% cover) is comparable to that estimated for short-tussock grasslands (10.8–27.2 $t \cdot ha^{-1}$ (Tate *et al.* 1997)), which is typically the adjacent vegetation cover in the high country. This result is somewhat puzzling given the obvious differences in standing biomass between woodland and grassland. However, the Tate *et al.* (1997) grassland figures were not based on any empirical data and therefore may have overestimated grassland carbon stocks (Carswell *et al.* 2008). The age and size class distributions of Birdwood Station and Mt Ben Ohau indicate that the majority of trees in both stands are relatively young (<100 years old). These are post-human disturbance stands and will thus continue to increase in biomass. As such, the figures given here are considered to underestimate the potential carbon stocks of these sites, as it is expected they will develop larger carbon stocks over time.

Carbon stocks within *Podocarpus cunninghamii* stands cannot be estimated solely on annual soil moisture deficit. Stands at both Mt Cook National Park and Mt Dobson, just like Upper Lawrence and Upper Hapuku, have no annual soil moisture deficits. However, the estimated carbon stocks of Mt Cook National Park and Mt Dobson are approximately five times lower. *Podocarpus cunninghamii* basal area and stocking density are also smaller at both these sites compared with Upper Lawrence and Upper Hapuku. The reasons for this are unclear, but the frequency and extent of large scale disturbance, as well as mean annual and winter minimum temperatures may play an

important role. For example, Mt Dobson and Upper Lawrence are floristically very similar, but their age and size class distributions show that Mt Dobson has far fewer individuals in the larger size classes. Mt Dobson is also populated by younger trees, indicating more recent disturbance and stand recovery (Chapter 3).

The estimated living carbon stocks within stands of *Podocarpus cunninghamii* do represent significant pools of carbon when compared with the grasslands that they typically occur within. Less disturbed forest stands contain higher quantities of carbon (e.g. Upper Lawrence > Mt Dobson > Birdwood Station). The rehabilitation of disturbed stands should therefore lead to the storage of even greater quantities of carbon.

10.4.2 Estimation of carbon sequestration rate

A preliminary model of annual *Podocarpus cunninghamii* diameter increment and carbon sequestration rates over a 100 year period in the high country has been developed. The model has several limitations and consequently the results should be interpreted with caution. The age data does not take into account the time it takes for *Podocarpus cunninghamii* to reach coring age. Resultantly, all age estimates must be considered as underestimates; this is a problem further compounded by missing rings (Bell & Bell 1959; Scott 1972). Tree ring counts are also heavily skewed to younger age classes (<60 years) due to rotten cores in larger trees, a higher frequency of missing the chronological centre of larger trees, and a paucity of larger trees in general. The data behind the model is therefore biased towards dryland samples, and as a result is likely to underestimate growth in the wetter parts of the high country. The growth data is also based on measurements of tree rings, which come from a single radius of each tree. Mean growth rate estimates should be based on repeated measurements over time (Kirschbaum *et al.* 2009). The rate of diameter growth also ignores density dependent effects arising from different stocking rates. Finally, an allometric equation specific to *Podocarpus cunninghamii* would improve biomass and carbon stock estimates. The allometric equation used here was taken from Coomes *et al.* (2002), which was based on data collected from a single, mixed beech/podocarp forest (Beets 1980).

The range of MAIs predicted by the model is within that recorded for the New Zealand Podocarpaceae (Hall & Hollinger 2000). The estimated annual carbon sequestration rates (0.1 and 0.4 $t \cdot ha^{-1} \cdot yr^{-1}$ for 250 and 1,000 $stems \cdot ha^{-1}$, respectively) are lower than the estimated mean national indigenous forest sequestration rate of 1.4 $t \cdot ha^{-1} \cdot yr^{-1}$ (Kirschbaum *et al.* 2009). However, the mean national rate includes areas of forest in mild and wet lowland environments more favourable for tree growth, includes faster growing tree species, and does not limit the number of potential species within indigenous forest to just one (Hall & Hollinger 2000). As such, it is not surprising that predicted *Podocarpus cunninghamii* sequestration rates are lower than the national mean.

The area of erosion-prone hill country on the South Island suitable for afforestion has been estimated at between 222,611 and 400,105 ha, of which the majority is described as low-producing or depleted grasslands (Davis *et al.* 2009). This represents a substantial area on which indigenous afforestation could take place. While not every hectare of this hill country is suitable for *Podocarpus cunninghamii*, for example *Nothofagus* may be more suited in some sites, the area available for *Podocarpus cunninghamii* restoration is still significant. Furthermore, the predicted 100 year carbon stock of a 1,000 $stems \cdot ha^{-1}$ *Podocarpus cunninghamii* stand (36 $t \cdot ha^{-1}$) exceeds the estimated range of carbon stock values for short tussock grassland. As such, afforestation of Kyoto-compliant high country grasslands with *Podocarpus cunninghamii* forest has the potential to increase New Zealand's carbon stocks, and thereby assist it achieve its Kyoto commitments in the medium to long term (Carswell *et al.* 2008; Kirschbaum *et al.* 2009).

In the short term, the estimated carbon sequestration rate of *Podocarpus cunninghamii* is much lower than that calculated for Radiata pine (*Pinus radiata*) (Davis *et al.* 2009). However, in the long term, indigenous forests have the potential to develop greater carbon stocks than conventionally managed exotic conifer forests (Carswell *et al.* 2008; Chazdon 2008), as well as provide greater benefits for associated indigenous flora and fauna. When combined with the estimated area of marginal land available for afforestation, these considerations augur favourably for the restoration of *Podocarpus cunninghamii* vegetation communities to the high country.

11 Conclusions

Prior to human arrival in New Zealand, the eastern South Island high country was a predominantly forested environment (McGlone, 1989). Sub-fossil log and charcoal remains and palynological deposits indicate that, outside of the areas dominated by *Nothofagus*, *Podocarpus cunninghamii* was a major forest component (McGlone, 2004, Molloy *et al.*, 1963). Early Polynesian fires followed by further forest clearance by European pastoralists caused widespread and massive deforestation, thereby creating the iconic high country tussock grasslands present today (Ewers *et al.*, 2006, McGlone, 2001). *Podocarpus cunninghamii* dominated vegetation communities still exist within the high country, but they tend to be only small remnants with a highly fragmented distribution (see Chapter 2). The overall aims of this thesis were to gain a better understanding of the ecology of these remnant high country *Podocarpus cunninghamii* dominated communities, and to investigate factors potentially affecting *Podocarpus cunninghamii* restoration within this environment, with special attention paid to the role of AMF.

In this concluding chapter, a summary of the main findings of this thesis is provided, together with some further discussion of the implications for restoration as well as some practical recommendations. Following this, areas of potential future research work are outlined before finally, the general conclusions of the thesis are presented.

11.1 The ecology of *Podocarpus cunninghamii*

11.1.1 Community ecology

The results of Chapter 3 showed that *Podocarpus cunninghamii* communities separate into two main types following a gradient of annual soil moisture deficit. Within this split, soil fertility factors and mean and minimum annual temperatures drive additional changes in floristic composition. At the wetter end of the scale, tall forest communities develop, with *Podocarpus cunninghamii* emergent over canopy species that typically include *Griselinia littoaralis*, *Pseudopanax colensoi* var. *ternatus* and *Phyllocladus alpinus*. *Coprosma linariifolia* is common in the sub-canopy and *Polystichum vestitum* is the dominant ground cover. Within the drylands,

Podocarpus cunninghamii communities have a much more open structure and rarely form a closed canopy. Canopy species are rare in the drylands and instead the community has a greater abundance of small tree species, e.g. *Hoheria lyallii* and *Pittosporum tenuifolium*, and shrubs, e.g. *Aristotelia fruticosa, Coprosma propinqua, Corokia cotoneaster* and *Discaria toumatou*. Exotic species are also common, particularly *Rosa rubiginosa*, *Ribes uva-crispa* and *Hieracium* species. The average canopy cover value of a *Podocarpus cunninghamii* community in the wetter parts of the high country is 90%, while in the drylands it is only 54%. Similarly, mean basal area and *Podocarpus cunninghamii* stem density of stands in the wetter parts of the high country are 25.06 $m^2 \cdot ha^{-1}$ and 215 $stems \cdot ha^{-1}$, respectively, while the corresponding figures for the dryland stands are 8.24 $m^2 \cdot ha^{-1}$ and 708 $stems \cdot ha^{-1}$.

In total, seven *Podocarpus cunninghamii* community types were identified based on floristic composition. Greatest species diversity was found in the types occurring at the wetter end of the annual soil moisture deficit gradient and the lowest diversity found at the drier end. The communities in the drylands, which are also smaller and more fragmented, support a greater abundance of exotic species, and these contribute substantially to their diversity indices. This relationship is a common feature of highly fragmented communities (Cook *et al.*, 2002, Ewers and Didham, 2006).

11.1.2 Population dynamics

The dendrochronological work undertaken in Chapter 3 demonstrated that dbh provides a reasonable indicator of approximate *Podocarpus cunninghamii* age (ages are approximate as it is not known how long it takes for *Podocarpus cunninghamii* to grow to coring height, and frequent missing rings means ring counts underestimate age). An individual increment core extracted from *Podocarpus cunninghamii* in the high country provides a 20-25% underestimate of the approximate minimum age of an individual tree. The amount of error associated with age estimates based on dbh size classes appeared relatively uniform across the high country. Thus the same level of uncertainty can be applied to age estimates from wet and dry stands.

The investigation into *Podocarpus cunninghamii* population dynamics indicated that *Podocarpus cunninghamii* has more than one regeneration strategy. Age and size

class distributions show that at some sites, *Podocarpus cunninghamii* undergoes continuous regeneration within intact forest. This suggests the presence of gap phase dynamics, whereby the regeneration is able to develop to the sapling stage but then requires some form of small-scale canopy disturbance, such as single tree fall, in order to attain canopy status. This process is similar to that described for *Podocarpus cunninghamii* in the North Island (Smale and Kimberley, 1993, Smale and Smale, 2003) and elsewhere on the South Island (Veblen and Stewart, 1982). In contrast, evidence from the Upper Hapuku, Seaward Kaikoura Range, suggests that *Podocarpus cunninghamii* regenerates via catastrophic disturbance, leading to the development of whole stands that are near even-aged. This research highlights the importance of disturbance regimes to maintain *Podocarpus cunninghamii* populations, and the need for restoration to incorporate disturbance processes.

11.1.3 Implications for restoration

In the wetter parts of the high country, stands of *Podocarpus cunninghamii* are relatively intact with few exotic species and evidence of ongoing canopy regeneration. The processes fundamental to forest survival are therefore being maintained. As such, these stands provide a clear species template for *Podocarpus cunninghamii* forest restoration within the wetter regions of the high country. The same cannot be said for the dryland stands. Stands in the drylands are highly degraded and are unlikely to be representative of the pre-human vegetation cover. In general terms, the pre-human dryland landscape below the alpine zone would have supported a low forest community, with emergent *Podocarpus cunninghamii* trees between 5-10 m in height. This community would have intergraded into a scrubland community with increasing soil moisture deficits. Species such as *Griselinia littoralis, Hoheria lyallii, Pseudopanax colensoi* var. *ternatus, Phyllocladus alpinus* and *Pittosporum tenuifolium* are all likely to have been common, with species such as *Aristotelia fruticosa, Corokia cotoneaster, Coprosma propinqua* and *Discaria toumatou* becoming more important with increasing soil moisture deficit.

Given the extent of pre-human forest loss in the high country, the simple removal of domestic livestock grazing is unlikely to result in the automatic return of *Podocarpus cunninghamii* communities. This is due to a sheer lack of seed sources as well as the

pervasive presence of feral herbivores (Norton, 2006, Standish *et al.*, 2009). As a result, restoration will be dependent on active human intervention. In addition, given the regime shifts that have occurred within the drylands since human arrival, it is likely that restoration will have to accept some level of exotic species being present (Walker *et al.*, 2009), and focussing resources on the least invaded/degraded remnants may provide the best opportunities for restoration. With that in mind, the classification system presented in Chapter 3 can be used as an indicator of community degradation and can therefore inform on restoration priorities.

11.2 AMF and *Podocarpus cunninghamii* restoration

11.2.1 Ex-agricultural and forest AMF

The literature review presented in Chapter 4 showed that there is increasing evidence that forest clearance and conversion to agriculture causes shifts in AMF community composition (Verchot, 2010). Subsequent agricultural practices can cause further changes, with the greatest change associated with increased agricultural intensification (Hijri *et al.*, 2006, Oehl *et al.*, 2003, Verbruggen *et al.*, 2010). These changes can potentially affect forest restoration on abandoned agricultural land.

Chapters 6 and 8 provided evidence that ex-agricultural (abandoned grazing grassland) AMF can have strong negative effects on the growth of *Podocarpus cunninghamii*, while AMF from a remnant stand of *Podocarpus cunninghamii* forest can have positive effects. Perhaps the most important result to come out of these chapters was that forest AMF can significantly improve the competitiveness of *Podocarpus cunninghamii* when grown in competition with grass, in both glasshouse and field settings. These results suggest that forest AMF have potential benefits for forest restoration on ex-agricultural land. Unfortunately the molecular work conducted in Chapter 5 was unable to elucidate the composition of the AMF communities of the forest or ex-agricultural soils. Without this information it is not possible to investigate how species composition affects the functional differences of the two AMF communities. Despite this, the results do add support to the work of Johnson (1993) and Ryan and Graham (2002), who found that agricultural practices can be associated with a decline in AMF mutualism. Soil fertiliser inputs tend to decrease plant root:shoot ratios and the transfer of carbohydrates from aboveground

plant tissues to belowground tissues (Hermans *et al.*, 2006). This can lead to a decrease in AMF mutualism by selecting for AMF that are more aggressive at acquiring carbon from their host (Graham and Abbott, 2000). The ex-agricultural AMF used in Chapter 6 were collected from a site retired from agriculture 16 years previously. This highlights legacy effects of forest clearance and agriculture, and demonstrates that reversing agriculturally driven AMF community changes is more difficult than simply removing grazing. The results also raised the issue of surrounding land use and the potential for AMF dispersal limitation to affect AMF community recovery following the cessation of agricultural practices.

11.2.2 Commercial and indigenous AMF

The results of Chapters 7 and 8 demonstrated that commercial AMF products hold no guarantee that they will improve plant growth and therefore assist ecological restoration, while indigenous AMF may. This adds to existing research that has presented similar results (Caravaca *et al.*, 2003, Rowe *et al.*, 2007). However, the results in no way suggest that all commercial AMF products will result in similar responses, and research has shown that indigenous AMF do not always result in positive native plant growth responses (Kiers *et al.*, 2000, Klironomos, 2003, Moora *et al.*, 2004). As such, more research trialling a wider range of AMF products and isolates is required.

The commercial AMF used in this thesis (Myco-Gro™, New Edge Microbials) was based on AMF extracted from Australian soils. This provided further evidence that AMF that are exotic to where they are subsequently applied may not be able to survive or provide plant benefit (Gianinazzi and Vosátka, 2004, Oliveira *et al.*, 2005). The introduction of exotic AMF to novel environments is also a cause for concern as introduced AMF have the potential to become invasive and the ecological consequences of their introduction is not yet known (Schwartz *et al.*, 2006). There is also evidence that foreign AMF can alter the performance of coexisting plant species compared with local AMF, which can have important implications for plant community composition and structure (Ji *et al.*, 2010, Yao *et al.*, 2008). This should be a serious concern for ecological restoration, and the evidence so far collected

suggests that, in general, indigenous AMF offer better prospects for restoration than commercially available products (Fuchs and Haselwandter, 2008).

11.2.3 Implications for restoration

The application of AMF to seedlings prior to outplanting has been shown to improve tree seedling growth and survival (Allen *et al.*, 2003, Allen *et al.*, 2005), and is considered an important tool in the process of restoring populations of endangered plant species (Bothe *et al.*, 2010). Chapter 8 provided additional evidence for this, as the application of *Acaulospora laevis* and forest AMF to *Podocarpus cunninghamii* prior to outplanting improved plant growth in the first growing season and indicated the potential for greater establishment success. However, data over successive growing seasons is required to validate this possibility. Previous research has demonstrated that improving the establishment success of planted *Podocarpus cunninghamii* seedlings in the high country is urgently needed (Ledgard, 2004).

The indigenous isolate of *Acaulospora laevis* used in Chapter 7 resulted in large increases in both *Podocarpus cunninghamii* growth and nutrient acquisition. However, grass competition cancelled all AMF effects. This suggests that the primary application of this AMF isolate is within native plant nurseries, where production times can potentially be reduced. At present it takes plant nurseries approximately two years (24 months) to produce a specimen of *Podocarpus cunninghamii* considered suitable for use in restoration (15-30 cm height). The use of *Acaulospora laevis* reduced the time taken to produce plants of this size to 15 months, thus reducing the production time by 9 months. Therefore, the use of *Acaulospora laevis* has the potential to decrease nursery turnover times of *Podocarpus cunninghamii* and thereby increase production. This should increase the quantity of *Podocarpus cunninghamii* seedlings available for restoration and reduce their cost. At present, individual seedlings of 15-30 cm height *Podocarpus cunninghamii* seedlings can cost over NZ$5, which can make restoration prohibitively expensive.

Further to this, it is worth noting that when *Podocarpus cunninghamii* was grown in the glasshouse without grass competition, forest AMF produced no noticeable effect. The effect of forest AMF only became evident once grass competition was added,

whether in a glasshouse environment (Chapter 6) or field setting (Chapter 8). On the otherhand, *Acaulospora laevis* had an immediate growth effect on *Podocarpus cunninghamii* when grown in the absence of grass in a glasshouse setting, but this effect disappeared with the addition of grass competition (Chapter 7). However, those seedlings treated with *Acaulospora laevis* showed significantly greater growth along with those treated with forest AMF once outplanted in the field (Chapter 8). These results highlight that AMF effects may not be immediately visible, i.e. in the nursery environment, but may have subsequent impacts on seedling growth and survival once outplanted.

The results of Chapter 9 indicate the New Zealand's native plant nurseries appreciate the potential for mycorrhizae to improve plant growth and establishment success, and many actively attempt to incorporate mycorrhizae into their propagation setups. However, their efforts may be going to waste due to a lack of understanding of the differences in biology of AMF and ectomycorrhizal fungi. Providing more information to native plant nurseries on the correct application of mycorrhizae is needed, and there is evidence that this beginning to happen (Corkidi *et al.*, 2008, Williams, 2009, Williams, 2010).

11.3 Carbon stocks and *Podocarpus cunninghamii* restoration

Chapter 10 provided first estimates of the carbon stocks present within several high country stands of *Podocarpus cunninghamii*. In general, carbon stocks are greater within the more intact stands in the wetter areas of the high country compared with the drylands. Tying this work in with that of Chapter 3, vegetation classes two and three (*Podocarpus cunninghamii* – (*Coprosma linariifolia*) forest and *Podocarpus cunninghamii* – (*Griselinia littoralis*) forest) hold the greatest carbon stocks (100.5 and 130.1 $t \cdot ha^{-1}$, respectively), while class 6 (*Podocarpus cunninghamii* – (*Coprosma propinqua*) treeland) holds the least carbon (7.3 $t \cdot ha^{-1}$). This is likely due to a combination of the dryland sites having lower standing biomass in general than the wetter sites, due to less favourable environmental conditions, and the dryland sites being more degraded, with a greater history of adverse anthropogenic impacts.

However, irrespective of vegetation class, *Podocarpus cunninghamii* stands hold greater carbon stocks than those estimated for grazing pastures (Tate *et al.*, 1997).

In addition, Chapter 10 presented a preliminary model of *Podocarpus cunninghamii* carbon sequestration rate. While this figure is much lower than the average rate estimated for New Zealand forests (0.4 $t \cdot ha^{-1} \cdot yr^{-1}$ for *Podocarpus cunninghamii* versus 1.4 $t \cdot ha^{-1} \cdot yr^{-1}$ for forests more generally (Kirschbaum *et al.*, 2009)), the estimated carbon sequestration rate of *Podocarpus cunninghamii* is still greater than that of grasslands. Although certain exotic pine species can grow faster than *Podocarpus cunninghamii* and therefore sequester greater quantities of carbon more rapidly, slower growing indigenous forests have the potential to develop greater carbon stocks in the long-term and enhance native biodiversity values (Carswell *et al.*, 2008, Chazdon, 2008). As such, the restoration of *Podocarpus cunninghamii* to the high country has the potential to assist New Zealand in achieving its commitments under the Kyoto Protocol. This provides some incentive for *Podocarpus cunninghamii* restoration above and beyond the well-established reasons of soil and indigenous biodiversity conservation.

11.4 Practical recommendations

Several practical recommendations relating to the restoration of *Podocarpus cunninghamii* communities to the high country can be drawn from this thesis. These can be separated into two areas: 1) where restoration seeks to create new *Podocarpus cunninghamii* forest where no *Podocarpus cunninghamii* forest currently exists; and 2) where restoration seeks to rehabilitate existing *Podocarpus cunninghamii* forest remnants. The recommendations for each follow. However, it must be kept in mind that these are tentative recommendations based on the information collected during the development of this thesis, and further research is required to confirm their efficacy or otherwise. In no way do they represent definitive rules for restoration.

11.4.1 Creating new *Podocarpus cunninghamii* forest

Given the cost of procuring large numbers of seedlings of native plants, especially podocarps, large-scale restoration of the high country to *Podocarpus cunninghamii* forest is, at present, unfeasible. Rather, restoration should focus on creating 'islands'

of *Podocarpus cunninghamii* forest on the most suitable land available, such as on erosion-prone marginal lands, in moist gullies, and in existing grey scrub communities. The successful creation of these islands will ensure a seed source for the natural colonisation of adjacent high country areas and provide much needed habitat for indigenous seed dispersers. Recommendations for the creation of these islands include:

- To ensure successful establishment and survival of palatable species, lagomorph and ungulate exclosure fencing should be erected.
- Further to this, control of mammalian herbivores could be assisted by encouraging recreational hunters to utilise hunting blocks in and around restoration sites.
- Grass cover should be reduced or removed through use of herbicides, mowing or mulching, to reduce competition with restoration plantings.
- Fast growing indigenous woody species should be planted initially. These will establish rapidly, shade-out grass and provide shelter for later successional species. Examples of such species include *Aristotelia fruticosa, Coprosma propinqua, Corokia cotoneaster* and *Hoheria lyallii*.
- Once these woody species are established, later successional species can be included, such as *Griselinia littoralis, Pseudopanax colensoi* var. *ternatus, Pseudopanax crassifolius, Sophora microphylla* and *Podocarpus cunninghamii*.
- Seedlings for restoration should also have been inoculated with appropriate AMF prior to outplanting. The results from this research suggest that indigenous AMF should be sourced from the top soil of remnant stands of *Podocarpus cunninghamii* forest.

11.4.2 Rehabilitating existing *Podocarpus cunninghamii* forest

Many existing high country *Podocarpus cunninghamii* communities offer good opportunities for ecological restoration. The problems that often face these sites are the prevalence of light-demanding exotic species, such as *Hieracium*, and the absence of more widespread woody species (Chapter 3). Reducing the prevalence of light-demanding exotic species can be achieved by increasing canopy cover. The reason

for the absence of widespread woody species is debatable, but for those that are highly palatable, such as *Griselinia littoralis*, mammalian herbivory is likely the cause. Recommendations for restoration include:

- Lagomorph and ungulate exclosure fencing should be erected.
- Highly palatable, shade-tolerant species should be reintroduced, for example *Griselinia littoralis*, *Pseudopanax colensoi* var. *ternatus* and *Pseudopanax crassifolius*.
- *Podocarpus cunninghamii* is an unpalatable species, and it is possible that livestock grazing assists its establishment by reducing the vigour of neighbouring grasses, while other more palatable woody species are excluded. Grassland areas adjacent to existing stands of *Podocarpus cunninghamii* should therefore be left unfenced to allow grazing to continue and encourage *Podocarpus cunninghamii* establishment. Once these seedlings become established, the area should then be fenced off to exclude grazing. *Podocarpus cunninghamii* can then itself act as a nurse plant and allow the species listed above and in 11.4.1 to be introduced.

11.5 Future research

11.5.1 A community approach to restoration

At the very beginning of this thesis, the restoration of *Podocarpus cunninghamii* forest was promulgated as a way of securing multiple ecosystem services, namely soil and indigenous biodiversity conservation, as well as carbon sequestration (Chapter 1). However, aside from Chapter 3, the thesis took a single species approach to restoration. This approach was taken for several reasons: 1) *Podocarpus cunninghamii* is the dominant and arguably most iconic species of these plant communities; 2) the restoration of a *Podocarpus cunninghamii*–type vegetation community will inherently rely upon the successful restoration of *Podocarpus cunninghamii*; and 3) *Podocarpus cunninghamii* represents the greatest biomass within these vegetation communities, and thus holds the majority of the carbon stocks. Despite this, there is accumulating evidence that for an ecosystem to provide multiple ecosystem services, increasing levels of biodiversity are required (Hector and Bagchi, 2007, Balvanera *et al.*, 2006). There is also accumulating evidence that

plant species interactions are strongly influenced by AMF species identity (Scheublin *et al.*, 2007), AMF community composition (Maherali and Klironomos, 2007, van der Heijden *et al.*, 1998b) and mycorrhizal networks (Selosse *et al.*, 2006, van der Heijden and Horton, 2009). The interaction between co-existing plant species and AMF therefore plays an important role in determining plant community composition (Stampe and Daehler, 2003, van der Heijden *et al.*, 1998a). In light of this, future research should take a more community-based approach to restoration and investigate the responses to different AMF inoculants of plant species combinations typical of *Podocarpus cunninghamii* vegetation communities. Such investigations should not be limited to establishing seedlings of multiple species at a single point in time solely within grasslands. They should also explicitly explore the impacts of existing mycorrhizal networks present within patches of seral woody vegetation in facilitating the establishment of *Podocarpus cunninghamii* forest seedlings.

11.5.2 Successional processes

Research in the tropics has demonstrated that forest AMF communities of individual tree species from the same location are significantly different depending on tree age (Husband *et al.*, 2002). In addition, tropical tree seedling growth responses have been shown to be greatest when inoculated with AMF from heterospecific adult trees rather than conspecific adult trees (Kiers *et al.*, 2000). AMF species present within mature forest may not be beneficial for tree seedlings (Allen *et al.*, 2003), although this may be dependent on soil organic matter content (Allen *et al.*, 2005). The forest AMF used in Chapters 6 and 8 was extracted from a mature stand of *Podocarpus cunninghamii*, and therefore suggests that mature forest AMF is beneficial for this species. However, future research should investigate the effect of AMF inoculants derived from *Podocarpus cunninghamii* forests at different stages of development, as well as AMF derived from seral high country woody communities. In addition, given that ecological restoration seeks to reinstate natural successional processes (SERI, 2004), identifying trajectories of *Podocarpus cunninghamii* community development based on AMF community type will be important.

11.5.3 Invasive species

The fitness of exotic plant species can be improved by certain AMF, which increases their potential to become invasive (Shah *et al.*, 2008, Desprez-Loustau *et al.*, 2007, Wolfe and Klironomos, 2005). There is also evidence that locally sourced AMF often improve the growth of native plants more than foreign AMF (e.g. Caravaca *et al.*, 2003, Ji *et al.*, 2010, Klironomos, 2003, Rowe *et al.*, 2007). In addition, the mycorrhizal symbiosis as a whole functions best (i.e. greater plant biomass, increased numbers of arbuscules and greater production of extraradical hyphae) when in association with native plants in home soil (Johnson *et al.*, 2010). This highlights the importance of local edaphic conditions and local plant-fungus combinations for optimising mycorrhizal function, and consequently for improving ecological restoration. However, invasive species can cause turnover in AMF community composition (Hawkes *et al.*, 2006). Such turnover would result in changes to mycorrhizal networks, which in turn may affect plant community structure and seedling establishment (Selosse *et al.*, 2006, van der Heijden and Horton, 2009). In light of this, future research should continue the work begun in Chapters 6 and 7, by investigating how competitive relationships of native and invasive plant species are influenced by different AMF inoculants. Of particular interest would be the response of *Hieracium*, which is highly invasive within the high country (Espie, 2001) and shows strong AMF dependency (Klironomos, 2003, van der Heijden *et al.*, 1998a).

11.5.4 Nursery production

Nursery inoculation of plants with AMF has great potential for assisting in plant production and ecological restoration (Bothe *et al.*, 2010, Jeffries *et al.*, 2003, Zhong *et al.*, 2010). However, there is a need, even with commercially available products, for AMF to be tested and matched to nursery production conditions (Corkidi *et al.*, 2004), as applications of fertiliser and fungicides to rooting mediums have been shown to reduce or inhibit mycorrhizae (Daft and Nicholson, 1969, O'Connor *et al.*, 2009). In addition, AMF do not necessarily result in increased plant growth within nurseries (Corkidi *et al.*, 2004). As such, the trade off between increased plant fitness and survival once field transplanted and decreasing plant production times needs to be investigated. AMF have also been shown to protect plants against root colonisation by fungal pathogens (Maherali and Klironomos, 2007, Sikes *et al.*, 2009). The

potential to reduce fungicide usage within nurseries therefore exists, but further research is required.

The application of indigenous AMF collected from existing native plant communities has been demonstrated to improve the growth of native plants (Allen *et al.*, 2005, Ji *et al.*, 2010, Klironomos, 2003, Requena *et al.*, 2001), which suggests they may play an important role in plant nurseries (Corkidi *et al.*, 2008, Williams, 2009). However, the use of field soils may introduce pathogens into nursery production systems. Furthermore, the effect on natural AMF and plant populations of repeated AMF collection are not known. Developing cultures of indigenous AMF may play an important role for native plant propagation (Corkidi *et al.*, 2008, Williams, 2010), by reducing soil disturbance to natural communities. However, pot cultures have been found to show selection for Glomeraceae species (Brundrett *et al.*, 1996), and as such further research is needed to determine how representative of natural communities these AMF cultures are, especially after several generations.

11.5.5 Carbon stocks

The allometric equation used in Chapter 10 to estimate aboveground biomass comes from a study from a single, mixed beech/podocarp forest in New Zealand (Beets, 1980, Coomes *et al.*, 2002). Estimates of *Podocarpus cunninghamii* biomass are therefore likely to carry high uncertainty. Determining whether *Podocarpus cunninghamii* belowground biomass equates to 0.25 of aboveground biomass, and whether the 0.5 conversion factor of biomass to carbon is a valid assumption for *Podocarpus cunninghamii*, is necessary to develop more reliable estimates of high country *Podocarpus cunninghamii* carbon stocks. A community based approach to estimating carbon stocks within *Podocarpus cunninghamii* stands is also recommended, and could be implemented based upon the community classification system presented in Chapter 3.

11.6 Final conclusions

The overall aims of this thesis were to gain a better understanding of the ecology of remnant high country *Podocarpus cunninghamii* dominated communities, and to investigate factors potentially affecting *Podocarpus cunninghamii* restoration within

this environment, with special attention paid to the role of AMF. The reason for this was to allow the development of a stronger ecological basis for future *Podocarpus cunninghamii* restoration in the high country. The findings of this thesis have shown that high country communities of *Podocarpus cunninghamii* show a high degree of floristic and structural variation based on soil and climatic variables. This variation must be taken into account when attempting to restore *Podocarpus cunninghamii* communities to the high country. Age and size class distributions indicate that *Podocarpus cunninghamii* tends to undergo continuous regeneration. Given that initial restoration plantings will most likely lead to the development of even-aged stands in the absence of human intervention, restoration may need to initiate small-scale canopy disturbances to allow the development of uneven aged stands that will then be able to regenerate without further management inputs.

The experimental data collected during this thesis suggest that AMF have a potentially important role to play in *Podocarpus cunninghamii* restoration. Firstly, AMF extracted from existing remnant stands of *Podocarpus cunninghamii* can improve the competitiveness of *Podocarpus cunninghamii* cuttings within a grassland environment in both glasshouse and field settings. The use of forest AMF may therefore be an important component of *Podocarpus cunninghamii* restoration within ex-agricultural grasslands, particularly as AMF extracted from ex-agricultural grasslands had a negative effect on *Podocarpus cunninghamii* growth. Secondly, in the absence of grass competition, the indigenous isolate of *Acaulospora laevis* was highly effective at increasing *Podocarpus cunninghamii* growth and nutrient acquisition. This suggests that this isolate may have a potentially important role to play in native plant nurseries by reducing the production time of *Podocarpus cunninghamii* plants for restoration. The diversity and potential applications of New Zealand's indigenous AMF communities are for the most part unknown. The results of this thesis indicate that these communities represent a largely untapped and potentially important resource for ecological restoration. However, to develop this resource, further investigation will be required to separate out those AMF that will limit or constrain restoration from those with the potential to assist and enhance it.

References

Abbott, L.K. & Robson, A.D. (1991) Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. *Agriculture, Ecosystems and Environment,* **35,** 121-150.

Al-Karaki, G., McMichael, B. & Zak, J. (2004) Field response of wheat to arbuscular mycorrhizal fungi and drought stress. *Mycorrhiza,* **14,** 263-269.

Alexander, T., Meier, R., Toth, R. & Weber, H.C. (1988) Dynamics of arbuscule development and degeneration in mycorrhizas of *Triticum aestivum* L. and *Avena sativa* L. with reference to *Zea mays* L. *New Phytologist,* **110,** 363-370.

Allen, E.B. & Allen, M.F. (1986) Water relations of xeric grasses in the field: interactions of mycorrhizas and competition. *New Phytologist,* **104,** 559-571.

Allen, E.B., Allen, M.F., Egerton-Warburton, L., Corkidi, L. & Gómez-Pompa, A. (2003) Impacts of early- and late-seral mycorrhizae during restoration in seasonal tropical forest, Mexico. *Ecological Applications,* **13,** 1701-1717.

Allen, E.B., Rincón, E., Allen, M.F., Pérez-Jimenez, A. & Huante, P. (1998) Disturbance and seasonal dynamics of mycorrhizae in a tropical deciduous forest in Mexico. *Biotropica,* **30,** 261-274.

Allen, M.F., Allen, E.B. & Gómez-Pompa, A. (2005) Effects of mycorrhizae and nontarget organisms on restoration of a seasonal tropical forest in Quintana Roo, Mexico: factors limiting tree establishment. *Restoration Ecology,* **13,** 325-333.

Aristizábal, C., Rivera, E.L. & Janos, D.P. (2004) Arbuscular mycorrhizal fungi colonize decomposing leaves of *Myrica parvifolia, M. pubescens* and *Paepalanthus* sp. *Mycorrhiza,* **14,** 221-228.

Artursson, V., Finlay, R.D. & Jansson, J.K. (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environmental Microbiology,* **8,** 1-10.

Asbjornsen, H. & Montagnini, F. (1994) Vesicular-arbuscular mycorrhizal inoculum potential affects the growth of *Stryphnodendron microstachyum* seedlings in a Costa Rican human tropical lowland. *Mycorrhiza,* **5,** 45-51.

Atkinson, I.A.E. (1985) Derivation of vegetation mapping units for an ecological survey of Tongariro National Park, North Island, New Zealand. *New Zealand Journal of Botany,* **23,** 361-378.

Augé, R.M. (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza,* **11,** 3-42.

Baeten, L., Hermy, M., Van Daele, S. & Verheyen, K. (2010) Unexpected understorey community development after 30 years in ancient and post-agricultural forests. *Journal of Ecology,* **98,** 1447-1453.

Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.-S., Nakashizuka, T., Raffaelli, D. & Schmid, B. (2006) Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters,* **9,** 1146-1156.

Baylis, G.T.S. (1959) Effect of vesicular-arbuscular mycorrhizas on growth of *Griselinia littoralis* (Cornaceae). *New Phytologist,* **58,** 274-278.

Baylis, G.T.S. (1967) Experiments on the ecological significance of phycomycetous mycorrhizas. *New Phytologist,* **66,** 231-243.

Baylis, G.T.S. (1969) Mycorrhizal nodules and growth of Podocarpus in nitrogen-poor soil. *Nature,* **223,** 1385-1386.

Baylis, G.T.S. (1972) Minimum levels of available phosphorous for non-mycorrhizal plants. *Plant and Soil,* **36,** 233-234.

Baylis, G.T.S. (1980) Mycorrhizas and the spread of beech. *New Zealand Journal of Ecology,* **3**.

Bedini, S., Avio, L., Argese, E. & Giovannetti, M. (2007) Effects of long-term land use on arbuscular mycorrhizal fungi and glomalin-related soil protein. *Agriculture, Ecosystems and Environment,* **120,** 463-466.

Beets, P.N. (1980) Amount and distribution of dry matter in a mature beech/podocarp community. *New Zealand Journal of Forestry Science,* **10,** 395-418.

Bell, V. & Bell, R.E. (1959) Dendrochronological studies in New Zealand. *Tree-ring Bulletin,* **22,** 7-11.

Benjamini, Y. & Yekutieli, D. (2001) The control of the false discovery rate in multiple testing under dependency. *The Annals of Statistics,* **29,** 1165-1188.

Bergin, D.O. (2003) Early performance of planted totara in comparison with other indigenous conifers. *New Zealand Journal of Forestry Science,* **33,** 205-224.

Bergin, D.O., Kimberley, M.O. & Low, C.B. (2008) Provenance variation in *Podocarpus totara* (D. Don): Growth, tree form and wood density on a coastal site in the north of the natural range, New Zealand. *Forest Ecology and Management,* **255,** 1367-1378.

Bleach, C.M., Cope, R.J., Jones, E.E., Ridgway, H.J. & Jaspers, M.V. (2008) Impact of mycorrhizal colonisation on grapevine establishment in *Cylindrocarpon* infested soil. *New Zealand Plant Protection,* **61,** 311-316.

Boddington, C.L. & Dodd, J.C. (2000a) The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. I. Field studies in an Indonesian ultisol. *Plant and Soil,* **218,** 137-144.

Boddington, C.L. & Dodd, J.C. (2000b) The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. II. Studies in experimental microcosms. *Plant and Soil,* **218,** 145-157.

Boerner, R.E.J., DeMars, B.G. & Leicht, P.N. (1996) Spatial patterns of mycorrhizal infectiveness of soils long a successional chronosequence. *Mycorrhiza,* **6,** 79-90.

Borowicz, V.A. (2001) Do arbuscular mycorrhizal fungi alter plant-pathogen relations? *Ecology,* **82,** 3057-3068.

Bothe, H., Turnau, K. & Regvar, M. (2010) The potential role of arbuscular mycorrhizal fungi in protecting endangered plants and habitats. *Mycorrhiza,* **20,** 445-457.

Bowen, M.E., McAlpine, C.A., House, A.P.N. & Smith, G.C. (2007) Regrowth forests on abandoned agricultural land: a review of their habitat values for recovering forest fauna. *Biological Conservation,* **140,** 273-296.

Brundrett, M., Bougher, N., Dell, B., Grove, T. & Malajczuk, N. (1996) *Working with Mycorrhizas in Forestry and Agriculture.* ACIAR Monograph 32. ACIAR, Canberra.

Buchanan, J. (1868) The botany of Otago. *Transactions of the New Zealand Institute,* **1,** 22-37.

Burrows, C.J., Randall, P. & Moar, N.T. (1993) Aranuian vegetation history of the Arrowsmith Range, Canterbury, New Zealand III. Vegetation changes in the Cameron, upper South Ashburton, and Paddle Hill Creek catchments. *New Zealand Journal of Botany,* **31,** 147-174.

Burrows, C.J. & Russell, J.B. (1990) Aranuian vegetation history of the Arrowsmith Range, Canterbury I. Pollen diagrams, plant macrofossils, and buried soils from Prospect Hill. *New Zealand Journal of Botany,* **28,** 323-345.

Callaway, R.M., Cipollini, D., Barto, K., Thelen, G.C., Hallett, S.G., Prati, D., Stinson, K. & Klironomos, J. (2008) Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology,* **89,** 1043-1055.

Caravaca, F., Barea, J.M., Palenzuela, J., Figueroa, D., Alguacil, M.M. & Roldán, A. (2003) Establishment of shrub species in a degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi. *Applied Soil Ecology,* **22,** 103-111.

Carswell, F.E., Burrows, L.E. & Mason, N.W.H. (2009) Above-ground carbon sequestration by early-succesional woody vegetation. A preliminary analysis. *Science for Conservation 297*. Department of Conservation, Wellington.

Carswell, F.E., Mason, N.W.H., Davis, M.R., Briggs, C.M., Clinton, P.W., Green, W., Standish, R.J., Allen, R.B. & Burrows, L.E. (2008) Synthesis of carbon stock information regarding conservation land. *Contract report for DOC (LC0708/071)*. Landcare Research, Lincoln.

Chazdon, R.L. (2008) Beyond deforestation: restoring forests and ecosystem services on degraded lands. *Science,* **320,** 1458-1460.

Clapp, J.P., Young, J.P.W., Merryweather, J.W. & Fitter, A.H. (1995) Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. *New Phytologist,* **130,** 259-265.

Cockayne, L. (1928) *The Vegetation of New Zealand.* Engelmann, Leipzig.

Connor, H.E. & Edgar, E. (1987) Name changes in the indigenous New Zealand flora, 1960-1986 and Nomina Nova IV, 1983-1986. *New Zealand Journal of Botany,* **25,** 115-170.

Cook, W.M., Lane, K.T., Foster, B.L. & Holt, R.D. (2002) Island theory, matrix effects and species richness patterns in habitat fragments. *Ecology Letters,* **5,** 619-623.

Coomes, D.A., Allen, R.B., Scott, N.A., Goulding, C. & Beets, P. (2002) Designing systems to monitor carbon stocks in forests and shrublands. *Forest Ecology and Management,* **164,** 89-108.

Corkidi, L., Allen, E.B., Merhaut, D., Allen, M.F., Downer, J., Bohn, J. & Evans, M. (2004) Assessing the infectivity of commercial mycorrhizal inoculants in plant nursery conditions. *Journal of Environmental Horticulture,* **22,** 149-154.

Corkidi, L., Evans, M. & Bohn, J. (2008) An introduction to propagation of arbuscular mycorrhizal fungi in pot cultures for inoculation of native plant nursery stock. *Native Plants Journal,* **9,** 29-38.

Cramer, V.A., Hobbs, R.J. & Standish, R.J. (2008) What's new about old fields? Land abandonment and ecosystem assembly. *Trends in Ecology & Evolution,* **23,** 104-112.

Crawley, M.J. (2007) *The R Book.* John Wiley & Sons Ltd, Chichester.

Crush, J.R. (1973) Significance of endomycorrhizas in tussock grassland in Otago, New Zealand. *New Zealand Journal of Botany,* **11,** 645-660.

Daft, M.J. & Nicholson, T.H. (1966) Effect of Endogone mycorrhiza on plant growth. *New Phytologist,* **65,** 343-350.

Daft, M.J. & Nicholson, T.H. (1969) Effect of Endogone mycorrhiza on plant growth II. Influence of soluble phosphate on endophyte and host in maize. *New Phytologist,* **68,** 945-952.

Daniell, T.J., Husband, R., Fitter, A.H. & Young, J.P.W. (2001) Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. *FEMS Microbiology Ecology,* **36,** 203-209.

Davis, M., Douglas, G., Ledgard, N., Palmer, D., Dhakal, B., Paul, T., Bergin, D., Hock, B. & Barton, I. (2009) Establishing indigenous forest on erosion-prone grassland: land areas, establishment methods, costs and carbon benefits. *Contract report for MAF (MAF POL 0809-11192)*, pp. 1-97. SCION, Rotorua.

Davis, M.B. & Shaw, R.G. (2001) Range shifts and adaptive responses to quaternary climate change. *Science,* **292,** 673-679.

Dawson, J. & Lucas, R. (2000) *Nature Guide to the New Zealand Forest.* A Godwit Book, Random House New Zealand, Auckland.

de Lange, P.J. & Rolfe, J.R. (2010) *New Zealand Indigenous Vascular Plant Checklist.* New Zealand Plant Conservation Network, Wellington.

De Lisle, J.F. & Browne, M.L. (1968) *The climate and weather of the Otago Region.* New Zealand Meteorological Service, Wellington.

Desprez-Loustau, M.-L., Robin, C., Buée, M., Courtecuisse, R., Garbaye, J., Suffert, F., Sache, I. & Rizzo, D.M. (2007) The fungal dimension of biological invasions. *Trends in Ecology & Evolution,* **22,** 472-480.

DOC (2010) Department of Conservation. Retrieved 13th September, 2010, from http://www.doc.govt.nz/conservation/land-and-freshwater/land/south-island-high-country/tenure-review/history/.

Downs, T.M. & Radford, I.J. (2005) Arbuscular mycorrhizal fungal colonisation of *Hieracium lepidulum* roots in experimental and field soil inoculated media. *New Zealand Journal of Botany,* **43,** 843-850.

Driver, J.D., Holben, W.E. & Rillig, M.C. (2005) Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry,* **37,** 101-106.

Duncan, R.P. (1989) An evaluation of errors in tree age estimates based on increment cores in kahikatea (*Dacrycarpus dacrydioides*). *New Zealand Journal of Natural Sciences,* **16,** 31-37.

Dunn, P.K. (2009) tweedie: Tweedie exponential family models. R-package version 1.6.8. http://CRAN.R-project.org/package=tweedie.

Dunwiddie, P.W. (1979) Dendrochronological studies of indigenous New Zealand trees. *New Zealand Journal of Botany,* **17,** 251-266.

Egerton-Warburton, L.M. & Allen, E.B. (2000) Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecological Applications,* **10,** 484-496.

Egerton-Warburton, L.M., Johnson, N.C. & Allen, E.B. (2007) Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grasslands. *Ecological Monographs,* **77,** 527-544.

Espie, P. (2001) *Hieracium in New Zealand: Ecology and Management.* . AgResearch Ltd, Mosgiel.

Espie, P.R., Hunt, J.E., Butts, C.A., Cooper, P.J. & Harrington, W.M.A. (1984) Mackenzie Ecological Region - New Zealand Protected Natural Area Programme. Department of Lands and Survey, Wellington.

Ewers, R.M. & Didham, R.K. (2006) Confounding factors in the detection of species responses to habitat fragmentation. *Biological Reviews,* **81,** 117-142.

Ewers, R.M., Kliskey, A.D., Walker, S., Rutledge, D., Harding, J.S. & Didham, R.K. (2006) Past and future trajectories of forest loss in New Zealand. *Biological Conservation,* **133,** 312-325.

Facelli, E., Smith, S.E., Facelli, J.M., Christophersen, H.M. & Smith, F.A. (2010) Underground friends or enemies: model plants help to unravel direct and indirect effects of arbuscular mycorrhizal fungi on plant competition. *New Phytologist,* **185,** 1050-1061.

Fischer, C.R., Janos, D.P., Perry, D.A., Linderman, R.G. & Sollins, P. (1994) Mycorrhiza inoculum potentials in tropical secondary succession. *Biotropica,* **26,** 369-377.

Fitter, A. (1997) Nutrient acquisition. *Plant Ecology (2nd ed.)* (ed M. J. Crawley), pp. 51-72. Blackwell Science, Oxford.

Fitter, A.H. (1991) Costs and benefits of mycorrhizas: implications for functioning under natural conditions. *Experientia,* **47,** 350-355.

Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N. & Snyder, P.K. (2005) Global consequences of land use. *Science,* **309,** 570-574.

Forsyth, D.M., Coomes, D.A., Nugent, G. & Hall, G.M.J. (2002) Diet and diet preferences of introduced ungulates (Order: Artiodactyla) in New Zealand. *New Zealand Journal of Zoology,* **29,** 323-343.

Fuchs, B. & Haselwandter, K. (2008) Arbuscular mycorrhiza of endangered plant species: potential impacts on restoration strategies. *Mycorrhiza: Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics* (ed A. Varma). Springer-Verlag, Berlin.

Fuller, G.D. & Conard, H.S. (1965) Plant Sociology: The Study of Plant Communities. Authorised English Translation of Pflanzensoziologie by Dr J. Braun-Blanquet. Hafner Publishing Company, Inc., New York.

Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhiza and rusts. *Molecular Ecology,* **2,** 113-118.

Gavito, M.E., Pérez-Castillo, D., González-Monterrubio, C.F., Vieyra-Hernández, T. & Martínez-Trujillo, M. (2008) High compatibility between arbuscular mycorrhizal fungal communities and seedlings of different land use types in a tropical dry ecosystem. *Mycorrhiza,* **19,** 47-60.

Gazey, C., Abbott, L.K. & Robson, A.D. (2004) Indigenous and introduced arbuscular mycorrhizal fungi contribute to plant growth in two agricultural soils from south-western Australia. *Mycorrhiza,* **14,** 355-362.

Gerdemann, J.W. (1964) The effect of mycorrhiza on the growth of maize. *Mycologia,* **56,** 342-349.

Gianinazzi, S. & Vosátka, M. (2004) Inoculum of arbuscular mycorrhizal fungi for production systems: science meets business. *Canadian Journal of Botany,* **82,** 1264-1271.

Gibbs, H.K., Brown, S., Niles, J.O. & Foley, J.A. (2007) Monitoring and estimating tropical forest carbon stocks: making REDD a reality. *Environmental Research Letters,* **2,** 1-13.

Giovannetti, M. & Mosse, B. (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist,* **84,** 489-500.

Gómez-Aparicio, L., Zamora, R., Gómez, J.M., Hódar, J.A., Castro, J. & Baraza, E. (2004) Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. *Ecological Applications,* **14,** 1128-1138.

Gosling, P., Hodge, A., Goodlass, G. & Bending, G.D. (2006) Arbuscular mycorrhizal fungi and organic farming. *Agriculture, Ecosystems and Environment,* **113,** 17-35.

Govindarajulu, M., Pfeffer, P.E., Jin, H., Abubaker, J., Douds, D.D., Allen, J.W., Bücking, H., Lammers, P.J. & Shachar-Hill, Y. (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature,* **435,** 819-823.

Graham, J.H. & Abbott, L.K. (2000) Wheat responses to aggressive and non-aggressive arbuscular mycorrhizal fungi. *Plant and Soil,* **220,** 207-218.

Green, J.J., Baddeley, J.A., Cortina, J. & Watson, C.A. (2005) Root development in the Mediterranean shrub *Pistacia lentiscus* as affected by nursery treatments. *Journal of Arid Environments,* **61,** 1-12.

Grime, J.P., Mackey, J.M.L., Hillier, S.H. & Read, D.J. (1987) Floristic diversity in a model system using experimental microcosms. *Nature,* **328,** 420-422.

Gross, N., Bagousse-Pinguet, Y.L., Liancourt, P., Urcelay, C., Catherine, R. & Lavorel, S. (2010) Trait-mediated effect of arbuscular mycorrhiza on the competitive effect and response of a monopolistic species. *Functional Ecology,* **24,** 1122-1132.

Grove, P. (1994) Hawkdun Ecological District. A Survey Report for the Protected Natural Areas Programme. Department of Conservation, Dunedin.

Grüner, I.G. (2003) *Comparative Ecology and Conservation of Rare Native Broom, Carmichaelia (Fabaceae), South Island, New Zealand*. Unpublished PhD thesis, University of Canterbury, Christchurch.

Grüner, I.G. & Norton, D.A. (2006) Herbivory by hares as a threat to the native brooms *Carmichaelia juncea* and *C. vexillata*. *New Zealand Journal of Ecology,* **30,** 261-265.

Hall, G.M.J. & Hollinger, D.Y. (2000) Simulating New Zealand forest dynamics with a generalized temperate forest gap model. *Ecological Applications,* **10,** 115-130.

Hall, G.M.J. & McGlone, M.S. (2006) Potential forest cover of New Zealand as determined by an ecosystem process model. *New Zealand Journal of Botany,* **44,** 211-232.

Hall, I.R. (1977) Species and mycorrhizal infections of New Zealand Endogonaceae. *Transactions of the British Mycological Society,* **68,** 341-356.

Harper, J.L. (1977) *Population Biology of Plants.* Academic Press, London.

Harrington, W.M.A., Cooper, P.J., Davis, C.M., Hagham, T.D. & Mason, C.R. (1986) Heron Ecological District - Arrowsmith, Hakatere, and Two Thumb Ecological Districts. Survey Report for the Protected Natural Areas Programme. Department of Lands and Survey, Wellington.

Harris, J. (2009) Soil microbial communities and restoration ecology: facilitators or followers? *Science,* **325,** 573-574.

Hart, M.M. & Reader, R.J. (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist,* **153,** 335-344.

Hart, M.M., Reader, R.J. & Klironomos, J.N. (2003) Plant coexistence mediated by arbuscular mycorrhizal fungi. *Trends in Ecology & Evolution,* **18,** 418-423.

Hartmann, H.T., Kester, D.E., Davies Jr, F.T. & Geneve, R.L. (1997) *Plant Propagation: Principles and Practice.* Prentice-Hall International, London.

Hawkes, C.V., Belnap, J., D'Antonio, C. & Firestone, M.K. (2006) Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. *Plant and Soil,* **281,** 369-380.

Hayman, D.S. & Mosse, B. (1971) Plant growth responses to vesicular-arbuscular mycorrhiza. *New Phytologist,* **70,** 19-27.

He, X.-H., Critchley, C. & Bledsoe, C. (2003) Nitrogen transfer within and between plants through common mycorrhizal networks (CMNs). *Critical Reviews in Plant Sciences,* **22,** 531-567.

Hector, A. & Bagchi, R. (2007) Biodiversity and ecosystem multifunctionality. *Nature,* **448,** 188-190.

Helgason, T., Daniell, T.J., Husband, R., Fitter, A.H. & Young, J.P.W. (1998) Ploughing up the wood-wide web? *Nature,* **394,** 431.

Hermans, C., Hammond, J.P., White, P.J. & Verbruggen, N. (2006) How do plants respond to nutrient shortage by biomass allocations. *Trends in Plant Science,* **11,** 610-617.

Hicks, D.L. (1995) Control of Soil Erosion on Farmland. *MAF Policy Technical Paper 95/4*. Ministry of Agriculture and Forestry, Wellington.

Higham, T., Anderson, A. & Jacomb, C. (1999) Dating the first New Zealanders: the chronology of Wairau Bar. *Antiquity,* **73,** 420-427.

Hijri, I., Sýkorová, Z., Oehl, F., Ineichen, K., Mäder, P., Wiemken, A. & Redecker, D. (2006) Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. *Molecular Ecology,* **15,** 2277-2289.

Hill, M.O. & Šmilauer, P. (2005) TWINSPAN for Windows version 2.3. Centre for Ecology and Hydrology & University of South Bohemia, Huntingdon & Ceske Budejovice.

Hobbs, R.J. & Norton, D.A. (2004) Ecological filters, thresholds, and gradients in resistance to ecosystem reassembly. *Assembly Rules and Restoration Ecology: Bridging the Gap between Theory and Practice* (eds V. M. Temperton, R. J. Hobbs, T. Nuttle & S. Halle), pp. 72-95. Island Press, Washington.

Hodge, A., Campbell, C.D. & Fitter, A.H. (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature,* **413,** 297-299.

Holl, K.D., Loik, M.E., Lin, E.H.V. & Samuels, I.A. (2000) Tropical montane forest restoration in Costa Rica: overcoming barriers to dispersal and establishment. *Restoration Ecology,* **8,** 339-349.

Holloway, J.T. (1954) Forests and climates in the South Island of New Zealand. *Transactions of the Royal Society of New Zealand,* **82,** 329-410.

Hurst, J.M. & Allen, R.B. (2007) *The Recce Method for Describing New Zealand Vegetation - Expanded Manual.* Version 4, Landcare Research, Lincoln.

Husband, R., Herre, E.A. & Young, J.P.W. (2002) Temporal variation in the arbuscular mycorrhizal communities colonising seedlings in a tropical forest. *FEMS Microbiology Ecology,* **42,** 131-136.

Husheer, S.W. (2007) Introduced red deer reduce tree regeneration in Pureora Forest, central North Island, New Zealand. *New Zealand Journal of Ecology,* **31,** 79-87.

Ishii, S. & Loynachan, E. (2004) Rapid and reliable DNA extraction techniques from trypan-blue stained mycorrhizal roots: comparison of two methods. *Mycorrhiza,* **14,** 271-275.

Jakobsen, I., Smith, S.E. & Smith, F.A. (2003) Function and diversity of arbuscular mycorrhizae in carbon and mineral nutrition. *Mycorrhizal Ecology* (eds M. G. A. v. d. Heijden & I. R. Sanders). Springer-Verlag, Berlin.

Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I.R. & Frossard, E. (2002) Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza,* **12,** 225-234.

Jasper, D.A., Abbott, L.K. & Robson, A.D. (1989a) Hyphae of a vesicular-arbuscular mycorrhizal fungus maintain infectivity in dry soil, except when the soil is disturbed. *New Phytologist,* **112,** 101-107.

Jasper, D.A., Abbott, L.K. & Robson, A.D. (1989b) Soil disturbance reduces the infectivity of external hyphae of vesicular-arbuscular mycorrhizal fungi. *New Phytologist,* **112,** 93-99.

Jayahandran, K. & Fisher, J. (2008) Arbuscular mycorrhizae and their role in plant restoration in native ecosystems. *Mycorrhizae: Sustainable Agriculture and Forestry* (eds Z. A. Siddiqui, M. S. Aktar & K. Futai), pp. 195-209. Springer Science + Business Media BV, Heidelberg.

Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K. & Barea, J.M. (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils,* **37,** 1-16.

Ji, B., Bentivenga, S.P. & Casper, B.B. (2010) Evidence for ecological matching of whole AM fungal communities to the local plant–soil environment. *Ecology,* **91,** 3037-3046.

Johansen, W., Jakobsen, I. & Jensen, E.S. (1992) Hyphal transport of ^{15}N-labelled nitrogen by a vesicular-arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. *New Phytologist,* **122,** 281-288.

Johnson, N.C. (1993) Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications,* **3,** 749-757.

Johnson, N.C., Graham, J.H. & Smith, F.A. (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist,* **135,** 575-585.

Johnson, N.C. & Wedin, D.A. (1997) Soil carbon, nutrients, and mycorrhizae during conversion of dry tropical forest to grassland. *Ecological Applications,* **7,** 171-182.

Johnson, N.C., Wilson, G.W.T., Bowker, M.A., Wilson, J.A. & Miller, R.M. (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences USA,* **107,** 2093-2098.

Johnson, P.N. (1977) Mycorrhizal endogonaceae in a New Zealand forest. *New Phytologist,* **78,** 161-170.

Jørgensen, B. (1997) *The Theory of Dispersion Models.* Chapman and Hall, London.

Kahiluoto, H., Ketoja, E., Vestberg, M. & Saarela, I. (2001) Promotion of AM utilization through reduced P fertilization 2. Field studies. *Plant and Soil,* **231,** 65-79.

Kerr, J.R. & Curran, M.D. (1996) Applications of polymerase chain reaction-single stranded conformational polymorphism to microbiology. *Journal of Clinical Pathology: Molecular Pathology,* **49,** 315-320.

Khurana, E. & Singh, J.S. (2001) Ecology of seed and seedling growth for conservation and restoration of tropical dry forest: a review. *Environmental Conservation,* **28,** 39-52.

Kiers, E.T., Lovelock, C.E., Krueger, E.L. & Herre, E.A. (2000) Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. *Ecology Letters,* **3,** 106-113.

Kiers, E.T. & van der Heijden, M.G.A. (2006) Mutualistic stability in the arbuscular mycorrhizal symbiosis: exploring hypotheses of evolutionary cooperation. *Ecology,* **87,** 1627-1636.

Kirschbaum, M., Trotter, C., Wakelin, S., Braisden, T., Curton, D., Dymond, J., Ghani, A., Jones, H., Deurer, M., Arnold, G., Beets, P., Davis, M., Hedley, C., Peltzer, D., Ross, C., Schipper, L., Sutherland, A., Wang, H., Beare, M., Clothier, B., Mason, N. & Ward, M. (2009) Carbon Stocks and Changes in

New Zealand's Soils and Forests, and Implications of Post-2012 Accounting Options for Land-Based Emissions Offsets and Mitigation Opportunities - Including Appendices. *Contract report for MAF (LC0708/174)*, pp. 1-457. Landcare Research, Lincoln.

Klironomos, J.N. (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology,* **84,** 2292-2301.

Koide, R.T. (1991) Density-dependent response to mycorrhizal infection in *Abutilon theophrasti* Medic. *Oecologia,* **85,** 389-395.

Koide, R.T. & Dickie, I.A. (2002) Effects of mycorrhizal fungi on plant populations. *Plant and Soil,* **244,** 307-317.

Koide, R.T. & Kabir, Z. (2000) Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. *New Phytologist,* **148,** 511-517.

Kothari, S.K., Marschner, H. & Römheld, V. (1990) Direct and indirect effects of VA mycorrhizal fungi and rhizosphere microorganisms on acquisition of mineral nutrients by maize (*Zea mays* L.) in a calcareous soil. *New Phytologist,* **116,** 637-645.

Krüger, M., Stockinger, H., Krüger, C. & Schüßler, A. (2009) DNA-based species level detection of Glomeromycota: one PCR primer set for all arbuscular mycorrhizal fungi. *New Phytologist,* **183,** 212-223.

Kubota, M. & Hyakumachi, M. (2004) Morphology and colonization preference of arbuscular mycorrhizal fungi in *Clethra barbinervis*, *Cucumis sativus*, and *Lycopersicon esculentum*. *Mycoscience,* **45,** 206-213.

Kunstler, G., Coomes, D.A. & Canham, C.D. (2009) Size-dependence of growth and mortality influence the shade tolerance of trees in a lowland temperate rain forest. *Journal of Ecology,* **97,** 685-695.

Kurien, B.T. & Scofield, R.H. (2002) Extraction of nucleic acid fragments from gels. *Analytical Biochemistry,* **302,** 1-9.

Leathwick, J.R. (1998) Are New Zealand's *Nothofagus* species in equilibrium with their environment? *Journal of Vegetation Science,* **9,** 719-732.

Leathwick, J.R. (2001) New Zealand's potential forest pattern as predicted from current species-environment relationships. *New Zealand Journal of Botany,* **39,** 447-464.

Leathwick, J.R. & Whitehead, D. (2001) Soil and atmospheric water deficits and the distribution of New Zealand's indigenous tree species. *Functional Ecology,* **15,** 233-242.

Ledgard, N.J. (2001) The spread of lodgepole pine (*Pinus contorta*, Dougl.) in New Zealand. *Forest Ecology and Management,* **141,** 43-57.

Ledgard, N.J. (2004) Establishing native species in the high country. *High Country Forestry*, pp. 2-4. Forest Research, Christchurch.

Lee, W.G. (1998) The vegetation of New Zealand - functional, spatial and temporal gaps. *Ecosystems, entomology and plants* (ed R. Lynch), pp. 91-101. The Royal Society of New Zealand Miscellaneous Series.

Legendre, P. & Gallagher, E.D. (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia,* **129,** 271-280.

Legendre, P. & Legendre, L. (1998) *Numerical Ecology.* Elsevier Science B.V., Amsterdam.

Lekberg, Y., Koide, R.T., Rohr, J.R., Aldrich-Wolfe, L. & Morton, J.B. (2007) Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. *Journal of Ecology,* **95,** 95-105.

Li, C., Qi, J., Feng, Z., Yin, R., Guo, B., Zhang, F. & Zou, S. (2010) Quantifying the effect of ecological restoration on soil erosion in China's Loess Plateau region: an application of the MMF approach. *Environmental Management,* **45,** 476-487.

Li, L.-F., Li, T. & Zhao, Z.-W. (2007a) Differences of arbuscular mycorrhizal fungal diversity and community between a cultivated land, an old field, and a never-cultivated field in a hot and arid ecosystem of southwest China. *Mycorrhiza,* **17,** 655-665.

Li, L.-F., Zhang, Y. & Zhao, Z.-W. (2007b) Arbuscular mycorrhizal colonisation and spore density across different land-use types in a hot and arid ecosystem, Southwest China. *Journal of Plant Nutrition and Soil Science,* **170,** 419-425.

Li, X. & Wilson, S.D. (1998) Facilitation among woody plants establishing in an old field. *Ecology,* **79,** 2694-2705.

Lioussanne, L., Jolicoeur, M. & St-Arnaud, M. (2009) Role of the modification in root exudation induced by arbuscular mycorrhizal colonization on the intraradical growth of *Phytophthora nicotianae* in tomato. *Mycorrhiza,* **19,** 443-448.

LRI (1992) *New Zealand Land Resource Inventory.* Landcare Research Ltd, Palmerston North.

MAF (2001) *Regional study: Canterbury.* Ministry of Agriculture and Forestry, Wellington.

MAF (2007) Forestry in a New Zealand Emissions Trading Scheme. Engagement Document. Ministry of Agriculture and Forestry, Wellington.

MAF (2009) A guide to Look-up Tables for the Emissions Trading Scheme. Ministry of Agriculture and Forestry, Wellington.

Maherali, H. & Klironomos, J.N. (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science,* **316,** 1746-1748.

Maldonado, J.D., Tainter, F.H., Skipper, H.D. & Lacher, T.E. (2000) Arbuscular mycorrhiza inoculum potential in natural and managed tropical montane soils in Costa Rica. *Tropical Agriculture,* **77,** 27-32.

Mark, A.F., Michel, P., Dickinson, K.J.M. & McLennan, B. (2009) The conservation (protected area) status of New Zealand's indigenous grasslands: an update. *New Zealand Journal of Botany,* **47,** 53-60.

Mathimaran, N., Ruh, R., Jama, B., Verchot, L., Frossard, E. & Jansa, J. (2007) Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. *Agriculture, Ecosystems and Environment,* **119,** 22-32.

Matson, P.A., Parton, W.J., Power, A.G. & Swift, M.J. (1997) Agricultural intensification and ecosystem properties. *Science,* **277,** 504-509.

McEwan, W.M. (1978) Ecological Regions and Districts of New Zealand. Department of Conservation, Wellington.

McGlone, M.S. (1989) The Polynesian settlement of New Zealand in relation to environmental and biotic changes. *New Zealand Journal of Ecology,* **12,** 115-129.

McGlone, M.S. (2001) The origin of the indigenous grasslands of southeastern South Island in relation to pre-human woody ecosystems. *New Zealand Journal of Ecology,* **25,** 1-15.

McGlone, M.S. (2004) *Vegetation history of the South Island high country.* Landcare Research, Lincoln.

McGlone, M.S. & Basher, L.R. (1995) The deforestation of the Upper Awatere Catchment, Inland Kaikoura Range, Marlborough, South Island, New Zealand. *New Zealand Journal of Ecology,* **19,** 53-66.

McGlone, M.S., Mark, A.F. & Bell, D. (1995) Late Pleistocene and Holocene vegetation history, Central Otago, South Island, New Zealand. *Journal of The Royal Society of New Zealand,* **25,** 1-22.

McGlone, M.S. & Moar, N.T. (1998) Dryland Holocene vegetation history, Central Otago and the Mackenzie Basin, South Island, New Zealand. *New Zealand Journal of Botany,* **36,** 91-111.

McGlone, M.S., Moar, N.T. & Meurk, C.D. (1997) Growth and vegetation history of alpine mires on the Old Man Range, Central Otago, New Zealand. *Arctic and Alpine Research,* **29,** 32-44.

McGlone, M.S., Salinger, M.J. & Moar, N.T. (1993) Paleovegetation studies of New Zealand's climate since the last glacial maximum. *Global Climates sine the Last Glacial Maximum* (eds H.E. Wright Jr., J. E. Kutzbach, T. Webb III, W. F. Ruddiman, F. A. Street-Perrott & P. J. Bartlein), pp. 294-317. University of Minnesota Press, Minneapolis.

McGlone, M.S., Turney, C.S.M. & Wilmshurst, J.M. (2004) Late-glacial and Holocene vegetation and climatic history of the Cass Basin, central South Island, New Zealand. *Quaternary Research,* **62,** 267-279.

McGlone, M.S. & Wilmshurst, J.M. (1999) Dating initial Maori environmental impact in New Zealand. *Quaternary International,* **59,** 5-16.

McKelvey, P.J. (1984) Provisional classification of South Island virgin indigenous forests. *New Zealand Journal of Forestry Science,* **14,** 151-178.

McLea, W.L. (1996) The late-Quaternary pollen record of south-east Nelson, South Island, New Zealand. *New Zealand Journal of Botany,* **34,** 523-538.

McSaveney, M.J. & Whitehouse, I.E. (1989) Anthropic erosion of mountain land in Canterbury. *New Zealand Journal of Ecology,* **12,** 151-163.

Merryweather, J. & Fitter, A. (1998) The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*. I. Diversity of fungal taxa. *New Phytologist,* **138,** 117-129.

Meurk, C.D. & Hall, G.M.J. (2006) Options for enhancing forest biodiversity across New Zealand's managed landscapes based on ecosystem modelling and spatial design. *New Zealand Journal of Ecology,* **30,** 131-146.

Meurk, C.D., Walker, S., Gibson, R.S. & Espie, P. (2002) Changes in vegetation states in grazed and ungrazed Mackenzie Basin grasslands, New Zealand, 1990-2000. *New Zealand Journal of Ecology,* **26,** 95-106.

Millenium Ecosystem Assessment (2005) *Ecosystems and human well-being: current state and trends: findings of the Condition and Trends Working Group.* Island Press.

Moar, N.T. (1971) Contributions to the Quaternary history of the New Zealand flora 6. Aranuian pollen diagrams from Canterbury, Nelson, and North Westland, South Island. *New Zealand Journal of Botany,* **9,** 80-145.

Moar, N.T. (1980) Late Otiran and early Aranuian grassland in central South Island. *New Zealand Journal of Ecology,* **3,** 4-12.

Molloy, B.P.J. (1989) The management of semi-natural areas: some factors to consider. *Management of New Zealand's Natural Estate; Occasional Publication No. 1* (ed D. A. Norton), pp. 67-71. New Zealand Ecological Society, Christchurch.

Molloy, B.P.J., Burrows, C.J., Cox, J.E., Johnston, J.A. & Wardle, P. (1963) Distribution of subfossil forest remains, eastern South Island, New Zealand. *New Zealand Journal of Botany,* **1,** 68-77.

Molloy, B.P.J., Hodder, R.A.C. & Cowiem, D. (1976) Joint Report: Ruataniwha Reserve - Mackenzie Basin. *Botany Division Report 130*. Department of Scientific and Industrial Research, Lincoln.

Moora, M., Öpik, M., Sen, R. & Zobel, M. (2004) Native arbuscular mycorrhizal fungal communities differentially influence the seedling performance of rare and common *Pulsatilla* species. *Functional Ecology,* **18,** 554-562.

Moorman, T. & Reeves, F.B. (1979) The role of endomycorrhizae in revegetation practices in the semi-arid west. II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. *American Journal of Botany*, **66,** 14-18.

Mosse, B. (1973) Plant growth responses to vesicular-arbuscular mycorrhiza. *New Phytologist,* **72,** 127-136.

Mummey, D.L., Antunes, P.M. & Rillig, M.C. (2009) Arbuscular mycorrhizal fungi pre-inoculant identity determines community composition in roots. *Soil Biology and Biochemistry*, **41,** 1173-1179.

Munro, D. (1868) On the leading features of the geographical botany of the provinces of Nelson and Marlborough, New Zealand. *Transactions of the New Zealand Institute,* **1,** 6-17.

Murcia, C. (1995) Edge effects in fragmented forests: implications for conservation. *Trends in Ecology & Evolution,* **10,** 58-62.

Newsham, K.K., Fitter, A.H. & Watkinson, A.R. (1995) Mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology*, **83,** 991-1000.

Newsome, P.F.J. (1987) The vegetative cover of New Zealand. *Water and Soil miscellaneous publication No. 112*. National Water and Soil Conservation Authority, Wellington.

Norton, D.A. (1983) Population dynamics of subalpine *Libocedrus bidwillii* forests in the Cropp River Valley, Westland, New Zealand. *New Zealand Journal of Botany,* **21,** 127-134.

Norton, D.A. (1986) Within-tree growth ring variability in three indigenous tree species. *Mauri Ora,* **13,** 13-21.

Norton, D.A. (2006) Managing high country landscapes into the future. *High Country Landscape Management Forum 2005 Proceedings* (ed R. S. Lough), pp. 97-103. Otago Regional Council, Dunedin.

Norton, D.A. (2009) Species invasions and the limits to restoration: learning from the New Zealand experience. *Science,* **325,** 569-571.

Norton, D.A. & Leathwick, J.R. (1990) The lowland vegetation pattern, south Westland, New Zealand 1. Saltwater Ecological Area. *New Zealand Journal of Botany,* **28,** 41-51.

Norton, D.A. & Ogden, J. (1987) Dendrochronology: a review with emphasis on New Zealand applications. *New Zealand Journal of Ecology,* **10,** 77-95.

Norton, D.A., Palmer, J.G. & Ogden, J. (1987) Dendroecological studies in New Zealand 1. An evaluation of tree age estimates based on increment cores. *New Zealand Journal of Botany,* **25,** 373-383.

Nugent, G., Fraser, K.W. & Sweetapple, P.J. (1997) Comparison of red deer and possum diets and impacts in podocarp-hardwood forest, Waihaha Catchment, Pureora Conservation Park. *Science for Conservation 50*. Department of Conservation, Wellington.

Nugent, G., Fraser, W. & Sweetapple, P. (2001) Top down or bottom up? Comparing the impacts of introduced arboreal possums and 'terrestrial' ruminants on native forests in New Zealand. *Biological Conservation,* **99,** 65-79.

NZPCN (2010) "*Podocarpus cunninghamii*". New Zealand Plant Conservation Network. Retrieved 30th April, 2010, from http://www.nzpcn.org.nz/flora_details.asp?ID=1174.

O'Connor, P., Manjarrez, M. & Smith, S.E. (2009) The fate and efficacy of benomyl applied to field soils to suppress activity of arbuscular mycorrhizal fungi. *Canadian Journal of Microbiology,* **55,** 901-904.

O'Loughlin, C. (2005) The protective role of trees in soil conservation. *New Zealand Journal of Forestry,* **49,** 9-15.

Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T. & Wiemken, A. (2003) Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Applied and Environmental Microbiology,* **69,** 2816-2824.

Oehl, F., Sieverding, E., Ineichen, K., Ris, E.-A., Boller, T. & Wiemken, A. (2005) Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. *New Phytologist,* **165,** 273-283.

Ogden, J. (1985) An introduction to plant demography with special reference to New Zealand trees. *New Zealand Journal of Botany,* **23,** 751-772.

Ogden, J., Basher, L. & McGlone, M.S. (1998) Fire, forest regeneration and links with early human habitation: evidence from New Zealand. *Annals of Botany,* **81,** 687-696.

Ogden, J. & Stewart, G.H. (1995) Community dynamics of the New Zealand conifers. *Ecology of the Southern Conifers* (eds N. J. Enright & R. S. Hill), pp. 81-119. Smithsonian Institution Press, Washington D.C.

Oksanen, J., Kindt, R., Legendre, P., O' Hara, B., Simpsons, G.L., Solymos, P., Stevens, H.H. & Wagner, H. (2009) vegan: Community Ecology Package. R package version 1.15.4. http://CRAN.R-project.org/package=vegan.

Oliveira, R.S., Vosátka, M., Dodd, J.C. & Castro, P.M.L. (2005) Studies on the diversity of arbuscular mycorrhizal fungi and the efficacy of two native isolates in a highly alkaline anthropogenic sediment. *Mycorrhiza,* **16,** 23-31.

Onguene, N.A. & Kuyper, T.W. (2005) Growth response of three native timber species to soils with different arbuscular mycorrhizal inoculum potentials in South Cameroon Indigenous inoculum and effect of addition of grass inoculum. *Forest Ecology and Management,* **210,** 283-290.

Orlovich, D.A. & Cairney, J.W.G. (2004) Ectomycorrhizal fungi in New Zealand: current perspectives and future directions. *New Zealand Journal of Botany,* **42,** 721-738.

Oyekanmi, E.O., Coyne, D.L., Fagade, O.E. & Osonubi, O. (2007) Improving root-knot nematode management on two soybean genotypes through the application of *Bradyrhizobium japonicum*, *Trichoderma pseudokoningii* and *Glomus mosseae* in full factorial combinations. *Crop Protection,* **26,** 1006-1012.

Padilla, F.M. & Pugnaire, F. (2006) The role of nurse plants in the restoration of degraded environments. *Frontiers in Ecology and the Environment,* **4,** 196-202.

Pascoe, R.M. (1983) *The climate and weather of Marlborough.* New Zealand Meteorological Service, Wellington.

Paul, T.S.H. & Ledgard, N.J. (2008) Effect of felled wilding pines on plant growth in high country grasslands. *New Zealand Plant Protection,* **61,** 105-110.

Payne, T. & Norton, D.A. (2010) Thresholds to restoration in degraded tussock grasslands: the influence of shade on soil moisture. *Unpublished manuscript*, pp. 1-15. School of Forestry, University of Canterbury, Christchurch.

Peng, S., Eissenstat, D.M., Graham, J.H., Williams, K. & Hodge, N.C. (1993) Growth depression in mycorrhizal citrus at high-phosphorous supply. *Plant Physiology,* **101,** 1063-1071.

Pérez, M. & Urcelay, C. (2009) Differential growth response to arbuscular mycorrhizal fungi and plant density in two wild plants belonging to contrasting functional types. *Mycorrhiza,* **19,** 517-523.

Picone, C. (2000) Diversity and abundance of arbuscular-mycorrhizal fungus spores in tropical forest and pasture. *Biotropica,* **32,** 734-750.

Pivato, B., Offre, P., Marchelli, S., Barbonaglia, B., Mougel, C., Lemanceau, P. & Berta, G. (2009) Bacterial effects on arbuscular mycorrhizal fungi and mycorrhiza development as influenced by the bacteria, fungi, and host plant. *Mycorrhiza,* **19,** 81-90.

Pocknall, D.T. (1982) Modern pollen spectra from mountain localities, South Island, New Zealand. *New Zealand Journal of Botany,* **20,** 361-371.

Pollock, M.L., Lee, W.G., Walker, S. & Forrester, G. (2007) Ratite and ungulate preferences for woody New Zealand plants: influence of chemical and physical traits. *New Zealand Journal of Ecology,* **31,** 68-78.

R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Ramos-Zapata, J.A., Orellana, R. & Allen, E.B. (2006) Establishment of *Desmoncus orthacanthos* Martius (Arecaceae): effect of inoculation with arbuscular mycorrhizae. *Revista de Biologia Tropical,* **54,** 65-72.

Read, D.J. (1998) Plants on the web. *Nature,* **396,** 22-23.

Read, D.J. (2003) Towards ecological relevance - progress and pitfalls in the path towards an understanding of mycorrhizal functions in nature. *Mycorrhizal Ecology* (eds M.G.A. van der Heijden & I. R. Sanders). Springer-Verlag, Berlin.

Reay, S.D. & Norton, D.A. (1999) Assessing the success of restoration planting in a temperate New Zealand forest. *Restoration Ecology,* **7,** 298-308.

Redecker, D. (2000) Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. *Mycorrhiza,* **10,** 73-80.

Redecker, D., Hijri, I. & Wiemken, A. (2003) Molecular identification of arbuscular mycorrhizal fungi in roots: perspectives and problems. *Folia Geobotanica,* **38,** 113-124.

Redecker, D., Morton, J.B. & Bruns, T.D. (2000) Molecular phylogeny of the arbuscular mycorrhizal fungi *Glomus sinuosum* and *Sclerocystis coremioides*. *Mycologia,* **92,** 282-285.

Redecker, D. & Raab, P. (2006) Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia,* **98,** 885-895.

Renker, C., Zobel, M., Öpik, M., Allen, M.F., Allen, E.B., Vosátka, M., Rydlová, J. & Buscot, F. (2004) Structure, dynamics, and restoration of plant communities: do arbuscular mycorrhizae matter? *Assembly Rules and Restoration Ecology: Bridging the Gap between Theory and Practice* (eds V. M. Temperton, R. J. Hobbs, T. Nuttle & S. Halle), pp. 189-229. Island Press, Washington.

Requena, N., Jeffries, P. & Barea, J.M. (1996) Assessment of natural mycorrhizal potential in a desertified semiarid ecosystem. *Applied and Environmental Microbiology,* **62,** 842-847.

Requena, N., Perez-Solis, E., Azcón-Aguilar, C., Jeffries, P. & Barea, J.M. (2001) Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. *Applied and Environmental Microbiology,* **67,** 495-498.

Rether, B., Delmas, G. & Laouedj, A. (1993) Isolation of polysaccharide-free DNA from plants. *Plant Molecular Biology Reporter,* **11,** 333-337.

Richter, B.S. & Stutz, J.C. (2002) Mycorrhizal inoculation of Big Sacaton: implications for grassland restoration of abandoned agricultural fields. *Restoration Ecology,* **10,** 607-616.

Rillig, M.C. & Mummey, D.L. (2006) Mycorrhizas and soil structure. *New Phytologist,* **171,** 41-53.

Rogers, G., Walker, S. & Lee, W.G. (2005) *The role of disturbance in dryland New Zealand: past and present.* Science for Conservation 258, Department of Conservation, Wellington.

Rogers, G.M., Walker, S., Basher, L.M. & Lee, W.G. (2007) Frequency and impact of Holocene fire in eastern South Island, New Zealand. *New Zealand Journal of Ecology,* **31,** 129-142.

Rooney, D., Kennedy, N., Deering, L., Gleeson, D. & Clipson, N. (2006) Effect of sheep urine deposition on the bacterial community structure in an acidic upland grassland soil. *Applied and Environmental Microbiology,* **72,** 7231-7237.

Rooney, D.C. & Clipson, N.J.W. (2009) Synthetic sheep urine alters fungal community structure in an upland grassland soil. *Fungal Ecology,* **2,** 36-43.

Rose, A.B., Basher, L.R., Wiser, S.K., Platt, K.H. & Lynn, I.H. (1998) Factors predisposing short-tussock grasslands to *Hieracium* invasion in Marlborough, New Zealand. *New Zealand Journal of Ecology,* **22,** 121-140.

Rosendahl, S. (2008) Communities, populations and individuals of arbuscular mycorrhizal fungi. *New Phytologist,* **178,** 253-266.

Rosendahl, S. & Stuckenbrock, E.H. (2004) Community structure of arbuscular mycorrhizal fungi in undisturbed vegetation revealed by analyses of LSU rDNA sequences. *Molecular Ecology,* **13,** 3179-3186.

Rousset, O. & Lepart, J. (1999) Shrub facilitation of *Quercus humilis* regneration in succession on calcareous grasslands. *Journal of Vegetation Science,* **10,** 493-502.

Rowe, H.I., Brown, C.S. & Claassen, V.P. (2007) Comparisons of mycorrhizal responsiveness with field soil and commercial inoculum for six native montane species and *Bromus tectorum*. *Restoration Ecology,* **15,** 44-52.

Ruiz-Lozano, J.M., Azcon, R. & Gomez, M. (1995) Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: physiological and nutritional plant responses. *Applied and Environmental Microbiology,* **61,** 456-460.

Russell, A.J., Bidartondo, M.I. & Butterfield, B.G. (2002) The root nodules of the Podocarpaceae harbour arbuscular mycorrhizal fungi. *New Phytologist,* **156,** 283-295.

Ryan, A.P. (1987) *The climate and weather of Canterbury, including Aorangi.* New Zealand Meteorological Service, Wellington.

Ryan, M.H. & Graham, J.H. (2002) Is there a role for arbuscular mycorrhizal fungi in production agriculture? *Plant and Soil,* **244,** 263-271.

Sanders, I.R. (2004) Plant and arbuscular mycorrhizal fungal diversity - are we looking at the relevant levels of diversity and are we using the right techniques? *New Phytologist,* **164,** 415-418.

Sanders, I.R., Alt, M., Groppe, K., Boller, T. & Wiemken, A. (1995) Identification of ribosomal DNA polymorphisms among and within spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. *New Phytologist,* **130,** 419-427.

Saunders, D.A., Hobbs, R.J. & Margules, C.R. (1991) Biological consequences of ecosystem fragmentation: a review. *Conservation Biology,* **5,** 18-32.

Schenck, N.C. & Kinloch, R.A. (1980) Incidence of mycorrhizal fungi on six field crops in monoculture on a newly cleared woodland site. *Mycologia,* **72,** 445-456.

Schenck, N.C. & Pérez, Y. (1990) *Manual for the identification of VA mycorrhizal fungi.* Synergistic Publications, Gainesville.

Scheublin, T.R., van Logtestijn, R.S.P. & van der Heijden, M.G.A. (2007) Presence and identity of arbuscular mycorrhizal fungi influence competitive interactions between plant species. *Journal of Ecology,* **95,** 631-638.

Schüβler, A., Schwarzott, D. & Walker, C. (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research,* **105,** 1413-1421.

Schwartz, M.W., Hoeksema, J.D., Gehring, C.A., Johnson, N.C., Klironomos, J.N., Abbott, L.K. & Pringle, A. (2006) The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecology Letters,* **9,** 501-515.

Schwieger, F. & Tebbe, C.C. (2000) Effect of field inoculation with *Sinorhizobium meliloti* L33 on the composition of bacterial communities in rhizospheres of a target plant (*Medicago sativa*) and a non-target plant (*Chenopodium album*) - linking of 16s rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria. *Applied and Environmental Microbiology,* **66,** 3556-3565.

Scott, D. (1972) Correlation between tree-ring width and climate in two areas in New Zealand. *Journal of The Royal Society of New Zealand,* **2,** 545-560.

Selosse, M.-A., Richard, F., He, X. & Simard, S.W. (2006) Mycorrhizal networks: *des liaisons dangereuses*? *Trends in Ecology & Evolution,* **21,** 621-628.

SERI (2004) *The SER International Primer on Ecological Restoration.* Society for Ecological Restoration International Science & Policy Working Group. www.ser.org & Tucson: Society for Ecological Restoration International.

Shah, M.A., Reshi, Z. & Rashid, I. (2008) Mycorrhizal source and neighbour identity differently influence *Anthemis cotula* L. invasion in the Kashmir Himalaya, India. *Applied Soil Ecology,* **40,** 330-337.

Shannon, C.E. (1948) A mathematical theory of communication. *Bell System Technical Journal,* **27,** 379-423.

Shannon, C.E. & Weaver, W. (1949) *The Mathematical Theory of Communication.* University of Illinois Press, Urbana.

Sikes, B.A., Cottenie, K. & Klironomos, J.N. (2009) Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *Journal of Ecology,* **97,** 1274-1280.

Simard, S.W., Durall, D. & Jones, M. (2003) Carbon and nutrient fluxes within and between mycorrhizal plants. *Mycorrhizal Ecology* (eds M. G. A. van der Heijden & I. R. Sanders), pp. 33-74. Springer-Verlag, Berlin.

Smale, M.C. & Kimberley, M.O. (1993) Regeneration patterns in montane conifer/broadleaved forest on Mt Pureora, New Zealand. *New Zealand Journal of Forestry Science,* **23,** 123-141.

Smale, M.C. & Smale, P.N. (2003) Dynamics of upland conifer/broadleaved forest at Waihaha, central North Island, New Zealand. *Journal of The Royal Society of New Zealand,* **33,** 509-528.

Smith, S.E. & Read, D.J. (2008) *Mycorrhizal Symbiosis.* Academic Press, London.

Smyth, G., Hu, Y. & Dunn, P.K. (2009) statmod: Statistical Modeling. R package version 1.4.0. http://CRAN.R-project.org/package=statmod.

Speight, R. (1910) The post-glacial climate of Canterbury. *Transactions of the New Zealand Institute,* **43,** 408-420.

Staddon, P.L., Ramsey, C.B., Ostle, N., Ineson, P. & Fitter, A.H. (2003) Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of ^{14}C. *Science,* **300,** 1138-1140.

Stampe, E.D. & Daehler, C.C. (2003) Mycorrhizal species identity affects plant community structure and invasion: a microcosm study. *Oikos,* **100,** 362-372.

Standish, R.J., Sparrow, A.D., Williams, P.A. & Hobbs, R.J. (2009) A state-and-transition model for the recovery of abandoned farmland in New Zealand. *New Models for Ecosystem Dynamics and Restoration* (eds R. J. Hobbs & K. N. Suding). Island Press, Washington D.C.

Steinberg, P.D. & Rillig, M.C. (2003) Differential decomposition of arbuscular mycorrhizal fungal hyphae and glomalin. *Soil Biology and Biochemistry,* **35,** 191-194.

Stephens, P.A. & Sutherland, W.J. (1999) Consequences of the Allee effect for behaviour, ecology and conservation. *Trends in Ecology & Evolution,* **14,** 401-405.

Stockinger, H., Walker, C. & Schüßler, A. (2009) '*Glomus intraradices* DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. *New Phytologist,* **183,** 1176-1187.

Stokes, M.A. & Smiley, T.L. (1968) *An Introduction to Tree-Ring Dating.* The University of Chicago Press, Chicago.

Suding, K.N. & Hobbs, R.J. (2009a) Models of ecosystem dynamics as frameworks for restoration ecology. *New Models for Ecosystem Dynamics and Restoration* (eds R. J. Hobbs & K. N. Suding). Island Press, Washington, DC.

Suding, K.N. & Hobbs, R.J. (2009b) Threshold models in restoration and conservation: a developing framework. *Trends in Ecology & Evolution,* **24,** 271-279.

Swaffield, S. & Hughey, K. (2001) The South Island high country of New Zealand: landscape challenges and future management. *Mountain Research and Development,* **21,** 320-326.

Sylvia, D., Alagely, A., Kent, D. & Mecklenburg, R. (1998) Mycorrhizae of landscape trees produced in raised beds and containers. *Journal of Aboriculture,* **24,** 308-314.

Sylvia, D.M. & Schenck, N.C. (1983) Application of superphosphate to mycorrhizal plants stimulates sporulation of phosphorous-tolerant vesicular-arbuscular mycorrhizal fungi. *New Phytologist,* **95,** 655-661.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) *MEGA4*: Molecular Evolutionary Genetics Analyser (MEGA) software version 4.0. *Molecular Biology and Evolution,* **24,** 1596-1599.

Tate, K.R., Giltrap, D.J., Claydon, J.J., Newsome, P.F., Atkinson, I.A.E., Taylor, M.D. & Lee, R. (1997) Organic carbon stocks in New Zealand's terrestrial ecosystems. *Journal of The Royal Society of New Zealand,* **27,** 315-335.

Tautz, D. & Renz, M. (1983) An optimized freeze-squeeze method for recovery of DNA fragments from agarose gels. *Analytical Biochemistry,* **132,** 14-19.

Taylor, N.H. & Pohlen, I.J. (1970) Soil Survey Method. New Zealand Soil Bureau Bulletin 25.

Tebbe, C.C. & Vahjen, W. (1993) Interference of humic acids and DNA extracted extracted directly from soil in detection and transformation of recombinant DNA from bacteria and a yeast. *Applied and Environmental Microbiology,* **59,** 2657-2665.

Travis, J.M.J. (2003) Climate change and habitat destruction: a deadly anthropogenic cocktail. *Proceedings of the Royal Society of London. B,* **270,** 467-473.

Turjaman, M., Tamai, Y., Sitepu, I.R., Santoso, E., Osaki, M. & Tawaraya, K. (2008) Improvement of early growth of two tropical peat-swamp forest tree species *Ploiarium alternifolium* and *Calophyllum hosei* by two arbuscular mycorrhizal fungi under greenhouse conditions. *New Forests,* **36,** 1-12.

Uibopuu, A., Moora, M., Saks, Ü., Daniell, T., Zobel, M. & Öpik, M. (2009) Differential effect of arbuscular mycorrhizal fungal communities from ecosystems along management gradient on the growth of forest understorey plant species. *Soil Biology and Biochemistry,* **41,** 2141-2146.

UNFCC (1998) *Kyoto Protocol to the United Nations Framework Convention on Climate Change.* United Nations.

Urgiles, N., Loján, P., Aguirrem, N., Blaschke, H., Günter, S., Stimm, B. & Kottke, I. (2009) Application of mycorrhizal roots improves growth of tropical tree seedlings in the nursery: a step towards reforestation with native species in the Andes of Ecuador. *New Forests,* **38,** 229-239.

van der Heijden, M.G.A. (2003) Arbuscular mycorrhizal fungi as a determinant of plant diversity: in search of underlying mechanisms and general principles. *Mycorrhizal Ecology* (eds M. G. A. van der Heijden & I. R. Sanders), pp. 243-265. Springer-Verlag, Berlin.

van der Heijden, M.G.A., Bardgett, R.D. & van Straalen, N.M. (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters,* **11,** 296-310.

van der Heijden, M.G.A., Boller, T., Wiemken, A. & Sanders, I.R. (1998a) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology,* **79,** 2082-2091.

van der Heijden, M.G.A. & Horton, T.R. (2009) Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology,* **97,** 1139-1150.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A. & Sanders, I.R. (1998b) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature,* **396,** 69-72.

Veblen, T.T. & Stewart, G.H. (1982) On the conifer regeneration gap in New Zealand: the dynamics of *Libocedrus bidwillii* stands on South Island. *Journal of Ecology,* **70,** 413-436.

Vellend, M. (2004) Parallel effects of land-use history on species diversity and genetic diversity of forest herbs. *Ecology,* **85,** 3043-3055.

Verbruggen, E., Röling, W.F.M., Gamper, H.A., Kowalchuk, G.A., Verhoef, H.A. & van der Heijden, M.G.A. (2010) Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytologist,* **186,** 968-979.

Verchot, L.V. (2010) Impacts of forest conversion to agriculture on microbial communities and microbial function. *Soil Biology and Agriculture in the Tropics* (ed P. Dion), pp. 45-63. Springer-Verlag, Berlin.

Walker, S. (2000) Post-pastoral changes in composition and guilds in a semi-arid conservation area, Central Otago, New Zealand. *New Zealand Journal of Ecology,* **24,** 123-137.

Walker, S., Cieraad, E., Monks, A., Burrows, L., Wood, J., Price, R., Rogers, G. & Lee, B. (2009) Long-term dynamics and rehabilitation of woody ecosystems

in dryland South Island, New Zealand. *New Models for Ecosystem Dynamics and Restoration* (eds R. J. Hobbs & K. N. Suding), pp. 99-111. Island Press, Washington, DC.

Walker, S., Lee, W.G. & Rogers, G.M. (2003) *The woody vegetation of Central Otago, New Zealand: its present and past distribution and future restoration needs.* Science for Conservation 226, Department of Conservation, Wellington.

Walker, S., Lee, W.G. & Rogers, G.M. (2004a) Pre-settlement woody vegetation of Central Otago, New Zealand. *New Zealand Journal of Botany,* **42,** 613-646.

Walker, S., Lee, W.G. & Rogers, G.M. (2004b) The woody vegetation of Central Otago, New Zealand. *New Zealand Journal of Botany,* **42,** 589-612.

Walker, S., Price, R. & Rutledge, D. (2008) *New Zealand's remaining indigenous cover: recent changes and biodiversity protection needs.* Science for Conservation 284, Department of Conservation, Wellington.

Wang, Y.Y., Vestberg, M., Walker, C., Hurme, T., Zhang, X. & Lindström, K. (2008) Diversity and infectivity of arbuscular mycorrhizal fungi in agricultural soils of the Sichuan Province of mainland China. *Mycorrhiza,* **18,** 59-68.

Ward, C.M., Bruce, D.L., Rance, B.D. & Roozen, D.A. (1994) Lindis, Pisa and Dunstan Ecological Districts - A Survey Report for the Protected Natural Areas Programme. Department of Conservation, Dunedin.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H. & Wall, D.H. (2004) Ecological linkages between aboveground and belowground biota. *Science,* **304,** 1629-1633.

Wardle, J. (1971) The forests and shrublands of the Seaward Kaikoura Range. *New Zealand Journal of Botany,* **9,** 269-292.

Wardle, J.A. (1984) *The New Zealand Beeches.* New Zealand Forest Service, Wellington.

Wardle, P. (1963) Evolution and distribution of the New Zealand flora, as affected by Quaternary climates. *New Zealand Journal of Botany,* **1,** 3-17.

Wardle, P. (1964) Facets of the distribution of forest vegetation in New Zealand. *New Zealand Journal of Botany,* **2,** 352-366.

Wardle, P. (1991) *Vegetation of New Zealand.* Cambridge University Press, Cambridge.

Wardle, P. (2001a) Distribution of native forest in the upper Clutha district, Otago, New Zealand. *New Zealand Journal of Botany,* **39,** 435-446.

Wardle, P. (2001b) Holocene forest fires in the upper Clutha district, Otago, New Zealand. *New Zealand Journal of Botany,* **39,** 523-542.

Warner, N.J., Allen, M.F. & MacMahon, J.A. (1987) Dispersal agents of vesicular-arbuscular mycorrhizal fungi in a disturbed arid ecosystem. *Mycologia,* **79,** 721-730.

Webb, T.H. (1992) *Soils of the Upper Waitaki Basin, South Island, New Zealand.* Scientific Report No. 3. Department of Scientific and Industrial Research.

Wells, J.A. (1972) Ecology of *Podocarpus hallii* in Central Otago, New Zealand. *New Zealand Journal of Botany,* **10,** 399-426.

Whipps, J.M. (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany,* **82,** 1198-1227.

White, T.J., Burns, T., S., L. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications* (eds M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White), pp. 315-322. Academic Press, San Diego.

Whitehouse, I.E. (1984) Erosion in the eastern South Island high country - a changing perspective. *Journal of the Tussock Grasslands & Mountain Lands Institute Review,* **42,** 3-23.

Williams, A. (2009) The use of mycorrhizal fungi to improve the propagation of native trees and shrubs: Part I Collection and incorporation. *Indigena*, pp. 9-11 (November issue). New Zealand Farm Forestry Association Indigenous Forest Section.

Williams, A. (2010) The use of mycorrhizal fungi to improve the propagation of native trees and shrubs: Part II Culturing. *Indigena*, pp. 1-2 (February issue). New Zealand Farm Forestry Association Indigenous Forest Section.

Williams, P.A. (1989) Vegetation of the Inland Kaikoura Range, Marlborough. *New Zealand Journal of Botany,* **27,** 201-220.

Williams, P.H. & Haynes, R.J. (1995) Effect of sheep, deer and cattle dung on herbage production and soil nutrient content. *Grass and Forage Science,* **50,** 263-271.

Wilson, H.D. (1976) *Vegetation of Mount Cook National Park New Zealand.* National Park Authority, Wellington.

Wolfe, B.E. & Klironomos, J.N. (2005) Breaking new ground: soil communities and exotic plant invasion. *BioScience,* **55,** 477-487.

Wright, S.F. & Upadhyaya, A. (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil,* **198,** 97-107.

Wu, Q.-S. & Xia, R.-X. (2006) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Journal of Plant Physiology,* **163,** 417-425.

Yao, Q., Zhu, H.-H., Hu, Y.-L. & Li, L.-Q. (2008) Differential influence of native and introduced arbuscular mycorrhizal fungi on growth of dominant and subordinate plants. *Plant Ecology,* **196,** 261-268.

Zhang, Y., Zhong, C.L., Chen, Y., Chen, Z., Jiang, Q.B., Wu, C. & Pinyopusarerk, K. (2010) Improving drought tolerance of *Casuarina equisetifolia* seedlings by arbuscular mycorrhizas under glasshouse conditions. *New Forests,* **40,** 261-271.

Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology,* **7,** 203-214.

Zheng, F.-L. (2006) Effect of vegetation changes on soil erosion on the Loess Plateau. *Pedosphere,* **16,** 420-427.

Zhong, C., Zhang, Y., Chen, Y., Jiang, Q., Chen, Z., Liang, J., Pinyopusarerk, K., Franche, C. & Bogusz, D. (2010) Casuarina research and applications in China. *Symbiosis,* **50,** 107-114.

Zhu, Y.-G. & Miller, R.M. (2003) Carbon cycling by arbuscular mycorrhizal fungi in soil-plant systems. *Trends in Plant Science,* **8,** 407-409.

Appendix 1

The percentage cover abundances of each species recorded within each class, and within each height stratum, as identified in the TWINSPAN analysis of chapter three. All values are the averages of each species within each height stratum for all plots within that class. Each class is provided in its own table.

Table A1.1 – Percentage cover for each species recorded in class 1: *Podocarpus cunninghamii – Phyllocladus alpinus* forest.
.: absent; •: <1% cover; ‡: ≥1%, <5% cover

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
PODcun	15.9	18.9	11.6
PSEcvt	5.1	23.5	5.5
PHYalp	‡	24.4	13.4
GRIlit	‡	14.4	9.8
LEPsco	‡	‡	.
HOHlya	•	•	•
DRAlon	•	7.4	‡
PSEcra	•	•	•
COPdum	.	6.8	5.4
COPpro	.	5.5	5.1
BRAbuc	.	‡	•
COPlin	.	‡	‡
GAUcra	.	‡	‡
HEBsub	.	‡	‡
PODniv	.	‡	‡
DRAuni	.	‡	•
HEBsal	.	‡	‡
PITten	.	‡	•
OLEnum	.	‡	‡
ARIfru	.	•	•
COPpse	.	•	‡
COPrug	.	•	•
MYRdiv	.	•	•
GAUant	.	•	•
MYRnum	.	•	‡
PHOcoo	.	•	•
RUBsch	.	•	•
POLves	.	.	6.1
BLEpen	.	.	‡

Table A1.1 continued

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
ASPric	.	.	‡
ASPter	.	.	‡
UNCcla	.	.	‡
CORcot	.	.	‡
ASPflb	.	.	‡
CHIcon	.	.	‡
PTEaus	.	.	•
BLEcap	.	.	•
MELalp	.	.	•
HIEpra	.	.	•
ANIhaa	.	.	•
MICpus	.	.	•
POAcol	.	.	•
SENbel	.	.	•
ACIaur	.	.	•
ACRcol	.	.	•
ASPfla	.	.	•
CARbil	.	.	•
Carex spp.	.	.	•
CELang	.	.	•
CLEfor	.	.	•
COPser	.	.	•
DIGpur	.	.	•
DIStou	.	.	•
GENcor	.	.	•
HEBtra	.	.	•
HIEpil	.	.	•
HOLlan	.	.	•
LEUfra	.	.	•
MYCmur	.	.	•
OLEcor	.	.	•
PIMore	.	.	•
UNCfus	.	.	•
VIOcun	.	.	•
CELcor	.	.	•
CREcap	.	.	•
EPIatr	.	.	•
LUZbvr	.	.	•
MUEaus	.	.	•

Table A1.2 – Percentage cover for each species recorded in class 2: *Podocarpus cunninghamii* – (*Coprosma linariifolia*) forest.
.: absent; •: <1% cover; ‡: ≥1%, <5% cover

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
PODcun	69.8	13.9	2.7
COPlin	‡	12.7	5.0
PHYalp	•	8.0	‡
GRIlit	‡	6.7	‡
CARser	‡	6.0	‡
PITten	•	‡	‡
PSEcra	•	5.4	‡
PSEcvt	•	‡	‡
RUBcis	•	5.9	‡
HEBtra	.	12.5	6.2
COPpro	.	11.2	6.6
COPdum	.	‡	‡
ASTpet	.	‡	5.6
PSEcol	.	‡	‡
HEBsub	.	•	•
MYRdiv	.	•	.
HOHlya	.	•	‡
COPpxr	.	•	•
CLEfor	.	•	‡
POLves	.	.	68.5
BLEcap	.	.	15.0
MYCmur	.	.	6.6
UNCcla	.	.	‡
ASPter	.	.	‡
BLEpen	.	.	‡
CYStas	.	.	‡
MICpus	.	.	‡
RANref	.	.	•
SENbel	.	.	•
ASPflb	.	.	•
ASPric	.	.	•
FUCexc	.	.	•
GENcor	.	.	•
PTEaus	.	.	•
ARIfru	.	.	•
CARdeb	.	.	•
EPIped	.	.	•
HIElep	.	.	•
NEMtri	.	.	•
POAnov	.	.	•

Table A1.2 continued

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
HIEpra	.	.	•
MYRnum	.	.	•
OLEcor	.	.	•
OLEnum	.	.	•
Carex spp.	.	.	•

Table A1.3 – Percentage cover for each species recorded in class 3: *Podocarpus cunninghamii* – (*Griselinia littoralis*) forest.
.: absent; •: <1% cover; ‡: ≥1%, <5% cover

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
PODcun	32.8	15.9	5.3
GRIlit	13.7	16.0	8.0
PHYalp	‡	8.3	5.1
PSEcra	‡	‡	‡
COPlin	‡	10.0	5.3
HOHlya	‡	‡	‡
PITten	•	‡	‡
RUBcis	•	‡	‡
MYRdiv	•	‡	‡
HEBsal	•	‡	•
PSEcvt	•	•	‡
RUBsch	•	‡	‡
COPpro	.	5.3	5.1
CORcot	.	‡	‡
HEBsub	.	‡	‡
COPdum	.	‡	‡
OLEavi	.	‡	•
RAUano	.	•	•
GAUant	.	•	•
PITano	.	•	•
DRAlon	.	•	•
PHOcoo	.	•	•
ARIfru	.	•	‡
CHIcon	.	•	•
COPpse	.	•	•
COPrig	.	•	•
HEBtre	.	•	•
MUEaus	.	•	•
PARcvt	.	•	•
DIGpur	.	•	•

Table A1.3 continued

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
POLves	.	.	23.2
MICpus	.	.	9.0
BLEcap	.	.	‡
BLEpen	.	.	‡
ASPric	.	.	‡
LAGstr	.	.	‡
UNCcla	.	.	‡
ASPter	.	.	‡
ASPflb	.	.	‡
MYCmur	.	.	‡
PYRele	.	.	‡
CARdeb	.	.	‡
HIElep	.	.	•
HIEpil	.	.	•
HIEpra	.	.	•
CYStas	.	.	•
HYMvil	.	.	•
MYRnum	.	.	•
URTinc	.	.	•
GALapa	.	.	•
SCHtri	.	.	•
GENcor	.	.	•
MELalp	.	.	•
ANTodo	.	.	•
CELcor	.	.	•
CLEmar	.	.	•
SENbel	.	.	•
ASPtri	.	.	•
HOLmol	.	.	•
ACAdum	.	.	•
ACIaur	.	.	•
ANIhaa	.	.	•
Carex spp.	.	.	•
CHIrig	.	.	•
GALpro	.	.	•
HYDmos	.	.	•
HYPmil	.	.	•
NEMtri	.	.	•
POAcol	.	.	•
PTEaus	.	.	•
SENwai	.	.	•
STEpar	.	.	•
VIOcun	.	.	•

Table A1.4 – Percentage cover for each species recorded in class 4: *Podocarpus cunninghamii* – (*Phyllocladus alpinus*) woodland.
.: absent; •: <1% cover; ‡: ≥1%, <5% cover

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
PODcun	37.7	37.3	20.0
PHYalp	7.3	16.7	10.9
HOHlya	‡	‡	•
MUEcom	•	‡	‡
HEBtra	.	7.8	6.1
PITten	.	5.5	‡
RIBuva	.	5.3	9.2
COPpro	.	5.2	‡
HEBsub	.	‡	‡
OLEnum	.	‡	‡
BRAmon	.	‡	‡
ROSrub	.	‡	‡
COPlin	.	‡	•
CARaus	.	‡	‡
ARIfru	.	‡	‡
DIStou	.	‡	•
PODniv	.	‡	‡
CORcot	.	‡	•
HEBodo	.	•	•
OZOlep	.	•	‡
CLEfor	.	•	‡
ACIgla	.	•	•
CARpet	.	•	.
CHIcon	.	•	•
PARcvt	.	•	•
PHOcoo	.	•	•
PSEcvt	.	•	•
HIElep	.	.	15.2
MYCmur	.	.	‡
HIEpra	.	.	‡
HIEpil	.	.	‡
ASPflb	.	.	‡
BLEpen	.	.	‡
GALapa	.	.	‡
FESrub	.	.	•
ASPric	.	.	•
MELalp	.	.	•
CYStas	.	.	•
DACglo	.	.	•
PITano	.	.	•

Table A1.4 continued

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
SCHtri	.	.	•
ACAcae	.	.	•
Vicia spp.	.	.	•
ASPter	.	.	•
ASPtri	.	.	•
HOLlan	.	.	•
HOLmol	.	.	•
CARbil	.	.	•
LUZulo	.	.	•
Myosotis spp.	.	.	•
POLves	.	.	•
ANIfil	.	.	•
Carex spp.	.	.	•
CELspe	.	.	•
COPpvl	.	.	•
CORkin	.	.	•
ECHvul	.	.	•
MYRnum	.	.	•
RAOsub	.	.	•
RUMace	.	.	•
ACAine	.	.	•
CARdeb	.	.	•
EPIatr	.	.	•
GERses	.	.	•
HYPper	.	.	•
HYPrad	.	.	•
NEMtri	.	.	•
POLneo	.	.	•
STEmed	.	.	•
STEpar	.	.	•
TRIrep	.	.	•
UNCfus	.	.	•
VERtha	.	.	•

Table A1.5 – Percentage cover for each species recorded in class 5: *Podocarpus cunninghamii – (Corokia cotoneaster*) scrubland.
.: absent; •: <1% cover; ‡: ≥1%, <5% cover

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
PODcun	18.9	31.9	18.0
PARcvt	•	‡	•
PITten	•	‡	•
SOPmic	•	‡	•
HOHlya	•	‡	‡
RUBsch	•	‡	‡
MUEcom	•	‡	8.0
MYRdiv	•	‡	•
PHYalp	•	•	•
CORcot	.	15.7	7.8
COPpro	.	14.7	8.0
HEBsub	.	11.1	7.3
MELalp	.	5.6	7.5
ARIfru	.	‡	‡
DIStou	.	‡	‡
ROSrub	.	‡	‡
COPrig	.	‡	‡
GRIlit	.	‡	•
PITano	.	‡	‡
PODniv	.	•	•
COPlin	.	•	•
CLEmar	.	•	‡
OLEnum	.	•	•
CARpet	.	•	•
OLEodo	.	•	•
MUEaus	.	•	.
PHOcoo	.	•	•
RIBuva	.	•	•
RUBcis	.	•	•
HIEpil	.	.	‡
HIEpra	.	.	‡
GALapa	.	.	‡
HOLlan	.	.	‡
HYPper	.	.	‡
FESnov	.	.	‡
HOLmol	.	.	‡
CYStas	.	.	•
ACAdum	.	.	•
ACIaur	.	.	•
CELcor	.	.	•

Table A1.5 continued

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
POAcol	.	.	•
CERglo	.	.	•
ASPric	.	.	•
CLEfor	.	.	•
CHIrig	.	.	•
GAUcra	.	.	•
HELint	.	.	•
BLEpen	.	.	•
Carex spp.	.	.	•
RUMace	.	.	•
ACRcol	.	.	•
AGRpet	.	.	•
LUZruf	.	.	•
ANIfil	.	.	•
ASPflb	.	.	•
HIElep	.	.	•
ANTodo	.	.	•
ASPtri	.	.	•
COPdum	.	.	•
DRAuni	.	.	•
HEBbuc	.	.	•
HYPrad	.	.	•
PIMpse	.	.	•
TARoff	.	.	•
TRIrep	.	.	•
VIOcun	.	.	•
ASPter	.	.	•
CRAsin	.	.	•
EPIatr	.	.	•
STEmed	.	.	•

Table A1.6 – Percentage cover for each species recorded in class 6: *Podocarpus cunninghamii – (Coprosma propinqua*) treeland.
.: absent; •: <1% cover; ‡: ≥1%, <5% cover

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
PODcun	24.0	45.6	28.7
PHYalp	•	‡	‡
RUBsch	•	‡	‡
COPpro	.	16.4	10.9
DIStou	.	10.9	6.8

Table A1.6 continued

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
ARIfru	.	5.4	‡
CORcot	.	5.3	‡
ROSrub	.	‡	‡
MUEcom	.	‡	‡
PODniv	.	‡	6.9
CARpet	.	‡	•
MELalp	.	‡	‡
RIBuva	.	•	‡
CARcra	.	•	•
HEBsub	.	•	•
GRIlit	.	•	•
CLEmar	.	•	•
PARcvt	.	•	.
CHIrig	.	•	.
COPdum	.	•	•
COPrig	.	•	•
HIEpil	.	.	8.8
FESnov	.	.	6.8
HIEpra	.	.	‡
HIElep	.	.	‡
HOLmol	.	.	‡
BLEpen	.	.	‡
POLves	.	.	‡
AGRpet	.	.	•
ACIaur	.	.	•
LEUfra	.	.	•
RUMace	.	.	•
GALapa	.	.	•
LUZruf	.	.	•
ACAine	.	.	•
ASPflb	.	.	•
RAOsub	.	.	•
HOLlan	.	.	•
GAUdvn	.	.	•
ORErig	.	.	•
ACRcol	.	.	•
PACnov	.	.	•
GAUcra	.	.	•
TRIrep	.	.	•
CERglo	.	.	•
HEBpim	.	.	•
OREram	.	.	•
ANAbel	.	.	•

Table A1.6 continued

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
ASPric	.	.	•
COPche	.	.	•
GERses	.	.	•
HEBbuc	.	.	•
POAcol	.	.	•
POAnov	.	.	•
SCHpau	.	.	•
CARdeb	.	.	•
MYCmur	.	.	•
VIOcun	.	.	•
HYPrad	.	.	•
BULang	.	.	•
COPpvl	.	.	•
SENwai	.	.	•
STEmed	.	.	•
TARoff	.	.	•
VERtha	.	.	•

Table A1.7 – Percentage cover for each species recorded in class 7: *Kunzea ericoides – Podocarpus cunninghamii* scrubland.
.: absent; •: <1% cover; ‡: ≥1%, <5% cover

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
KUNeri	7.9	47.4	35.5
PODcun	7.4	25.5	18.0
COPpro	.	6.4	5.1
GAUant	.	‡	‡
DIStou	.	‡	‡
COPrig	.	‡	‡
CORcot	.	‡	‡
COPdum	.	‡	‡
CARpet	.	‡	‡
COPpse	.	‡	‡
DRAlon	.	‡	‡
ROSrub	.	•	‡
RUBsch	.	•	•
HIElep	.	.	21.1
MELalp	.	.	‡
HOLmol	.	.	‡
POAcol	.	.	‡

Table A1.7 continued

Species	Height strata		
	>3 m	**0.5 - 3 m**	**<0.5 m**
DACglo	.	.	‡
AGRcap	.	.	‡
RUMace	.	.	‡
ACRcol	.	.	‡
ACIaur	.	.	‡
PODniv	.	.	‡
PTEesc	.	.	‡
FESnov	.	.	•
LEUfra	.	.	•
LUZruf	.	.	•
ACAcae	.	.	•
ASPter	.	.	•
BLEpen	.	.	•
HOLlan	.	.	•
HYPper	.	.	•
MUEcom	.	.	•
RIBuva	.	.	•
TRIrep	.	.	•
ASPric	.	.	•
GERses	.	.	•
HYPrad	.	.	•
TRIarv	.	.	•

Appendix 2

The full scientific species names of the National Vegetation Survey (NVS) codes used in Figure 3.3 (chapter 3) and Appendix 1.

NVS code	Species name
ACAcae	*Acaena caesiglauca*
ACAdum	*Acaena dumicola*
ACAine	*Acaena inermis*
ACIaur	*Aciphylla aurea*
ACIcre	*Aciphylla crenulata*
ACIgla	*Aciphylla glaucescens*
ACRcol	*Acrothamnus colensoi*
AGRcap	*Agrostis capillaris*
AGRpet	*Agrostis petriei*
ANAbel	*Anaphalioides bellidioides*
ANIfil	*Anisotome filifolia*
ANIhaa	*Anisotome haastii*
ANTodo	*Anthoxanthum odoratum*
ARIfru	*Aristotelia fruticosa*
ASPfla	*Asplenium flaccidum*
ASPflb	*Asplenium flabellifolium*
ASPhoo	*Asplenium hookerianum*
ASPric	*Asplenium richardii*
ASPter	*Asplenium terrestre*
ASPtri	*Asplenium trichomanes*
ASTpet	*Astelia petriei*
BLEmon	*Blechnum montanum*
BLEpen	*Blechnum penna-marina*
BRAbuc	*Brachyglottis buchananii*
BRAcas	*Brachyglottis cassinioides*
BRAmon	*Brachyglottis monroi*
BULang	*Bulbinella angustifolia*
CARaus	*Carmichaelia australis*
CARbil	*Cardamine bilobata*
CARcra	*Carmichaelia crassicaulis*
CARdeb	*Cardamine debilis*
Carex spp.	Unidentifed *Carex* spp.
CARpet	*Carmichaelia petriei*
CARser	*Carpodetus serratus*
CELang	*Celmisia angustifolia*
CELcor	*Celmisia coriacea*
CELspe	*Celmisia spectabilis*

CERglo	*Cerastium glomeratum*
CHIcon	*Chionochloa conspicua*
CHIrig	*Chionochloa rigida*
CLEfor	*Clematis forsteri*
CLEmar	*Clematis marata*
COPche	*Coprosma cheesemanii*
COPdum	*Coprosma dumosa*
COPlin	*Coprosma linariifolia*
COPpro	*Coprosma propinqua*
COPpse	*Coprosma pseudocuneata*
COPpvl	*Coprosma propinqua* var. *latiuscula*
COPpxr	*Coprosma propinqua* × *robusta*
COPrig	*Coprosma rigida*
COPrug	*Coprosma rugosa*
COPser	*Coprosma serrulata*
CORcot	*Corokia cotoneaster*
CORkin	*Coriaria kingiana*
CRAsin	*Crassula sinclairii*
CREcap	*Crepis capillaris*
CYSfra	*Cystopteris fragilis*
CYStas	*Cystopteris tasmanica*
DACglo	*Dactylis glomerata*
DIGpur	*Digitalis purpurea*
DIStou	*Discaria toumatou*
DRAlon	*Dracophyllum longifolium*
DRAuni	*Dracophyllum uniflorum*
ECHvul	*Echium vulgare*
EPIatr	*Epilobium alsinoides* subsp. *atriplicifolium*
EPIped	*Epilobium pedunculare*
FESnov	*Festuca novae-zelandiae*
FESrub	*Festuca rubra*
FUCexc	*Fucshia excorticata*
GALapa	*Galium aparine*
GALpro	*Galium propinquum*
GAUant	*Gaultheria antipoda*
GAUcra	*Gaultheria crassa*
GAUdvn	*Gaultheria depressa* var. *novae-zelandiae*
GENcor	*Gentiana corymbifera*
GERses	*Geranium sessiliflorum*
GRIlit	*Griselinia littoralis*
HEBbuc	*Hebe buchananii*
HEBodo	*Hebe odora*
HEBpim	*Hebe pimeleoides*
HEBsal	*Hebe salicifolia*
HEBsub	*Hebe subalpina*
HEBtra	*Hebe traversii*

HEBtre	*Hebe treadwellii*
HELint	*Helichrysum intermedium*
HIElep	*Hieracium lepidulum*
HIEpil	*Hieracium pilosella*
HIEpra	*Hieracium praeltum*
HOHlya	*Hoheria lyallii*
HOLlan	*Holcus lanatus*
HOLmol	*Holcus mollis*
HYDmos	*Hydrocotyle moschata*
HYMvil	*Hymenophyllum villosum*
HYPmil	*Hypolepis millefolium*
HYPper	*Hypericum perforatum*
HYPrad	*Hypochoeris radicata*
KUNeri	*Kunzea ericoides*
LAGstr	*Lagenifera strangulata*
LEPsco	*Leptospermum scoparium*
LEUfra	*Leucopogon fraseri*
LUZbvr	*Luzula banksiana* var. *rhadina*
LUZruf	*Luzula rufa*
LUZulo	*Luzula ulophylla*
MELalp	*Melicytus alpinus*
MICpus	*Microsorum pustulatum*
MUEaus	*Muehlenbeckia australis*
MUEcom	*Muehlenbeckia complexa*
MYCmur	*Mycelis muralis*
Myosotis spp.	Unidentified *Myosotis* spp.
MYRdiv	*Myrsine divaricata*
MYRnum	*Mysine nummularia*
NEMtri	*Nematoceras trilobum*
OLEavi	*Olearia avicenniifolia*
OLEcor	*Olearia coriacea*
OLEnum	*Olearia nummulariifolia*
OLEodo	*Olearia odorata*
OREram	*Oreomyhrris ramosa*
OREram	*Oreomyrrhis ramosa*
ORErig	*Oreomyhrris rigida*
OZOlep	*Ozothamnus leptophyllus*
PACnov	*Pachycladon novae-zelandiae*
PARcvt	*Parsonsia capsularis* var. *tenuis*
PHOcoo	*Phormium cookianum*
PHYalp	*Phyllocladus alpinus*
PIMore	*Pimelea oreophila*
PIMpse	*Pimelea pseudolyallii*
PITano	*Pittosporum anomalum*
PITten	*Pittosporum tenuifolium*
POAcol	*Poa colensoi*

POAnov	*Poa novae-zelandiae*
PODcun	*Podocarpus cunninghamii*
PODniv	*Podocarpus nivalis*
POLneo	*Polystichum neozelandicum*
POLves	*Polystichum vestitum*
PSEcol	*Pseudowintera colorata*
PSEcra	*Pseudopanax crassifolius*
PSEcvt	*Pseudopanax colensoi* var. *ternatus*
PTEaus	*Pterostylis australis*
PTEesc	*Pteridium esculentum*
PYRele	*Pyrrosia eleagnifolia*
RANref	*Ranunculus reflexus*
RAOsub	*Raoulia subsericea*
RAUano	*Raukaua anomulus*
RIBuva	*Ribes uva-crispa*
ROSrub	*Rosa rubiginosa*
RUBcis	*Rubus cissoides*
RUBsch	*Rubus schmidelioides*
RUMace	*Rumex acetosella*
SCHpau	*Schoenus pauciflorus*
SCHtri	*Schizeilema trifoliolatum*
SENbel	*Senecio bellidioides*
SENwai	*Senecio wairauensis*
SOPmic	*Sophora microphylla*
STEmed	*Stellaria media*
STEpar	*Stellaria parviflora*
TARoff	*Taraxacum officinale*
TRIarv	*Trifolium arvense*
TRIrep	*Trifolium repens*
UNCcla	*Uncinia clavata*
UNCfus	*Uncinia fuscovaginata*
URTinc	*Urtica incisa*
VERtha	*Verbascum thapsus*
VICIA spp.	Unidentified *Vicia* spp.
VIOcun	*Viola cunninghamii*

Appendix 3

The neighbour joining distance trees of obtained AMF sequences against GenBank accessions. The maximum allowable fraction of mismatched bases was set at the BLAST default of 0.75.

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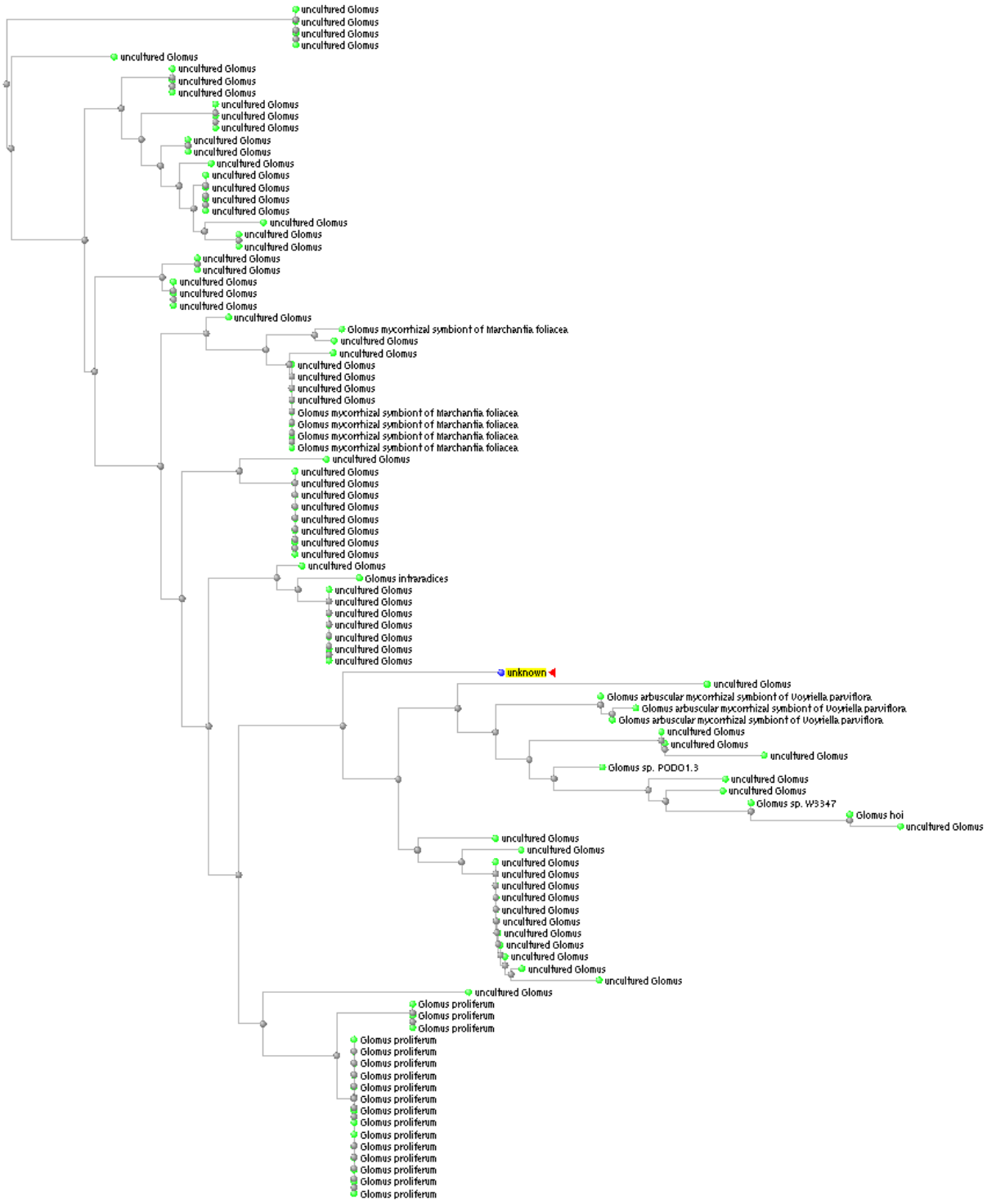

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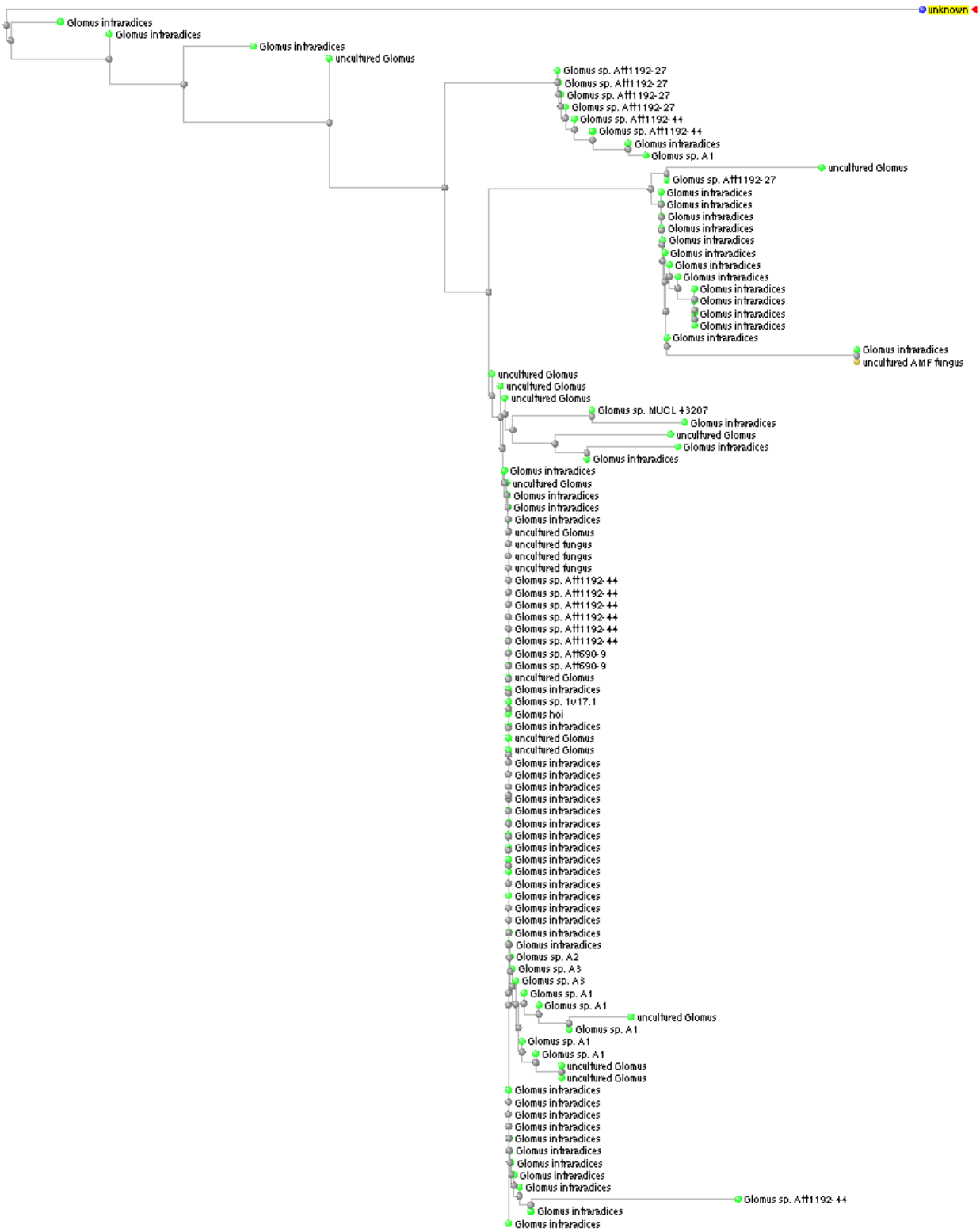
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Glomus sp. Att1192-27
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Glomus intraradices
uncultured AMF fungus
uncultured Glomus
uncultured Glomus
uncultured Glomus
Glomus sp. MUCL 43207
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uncultured Glomus
uncultured fungus
uncultured fungus
uncultured fungus
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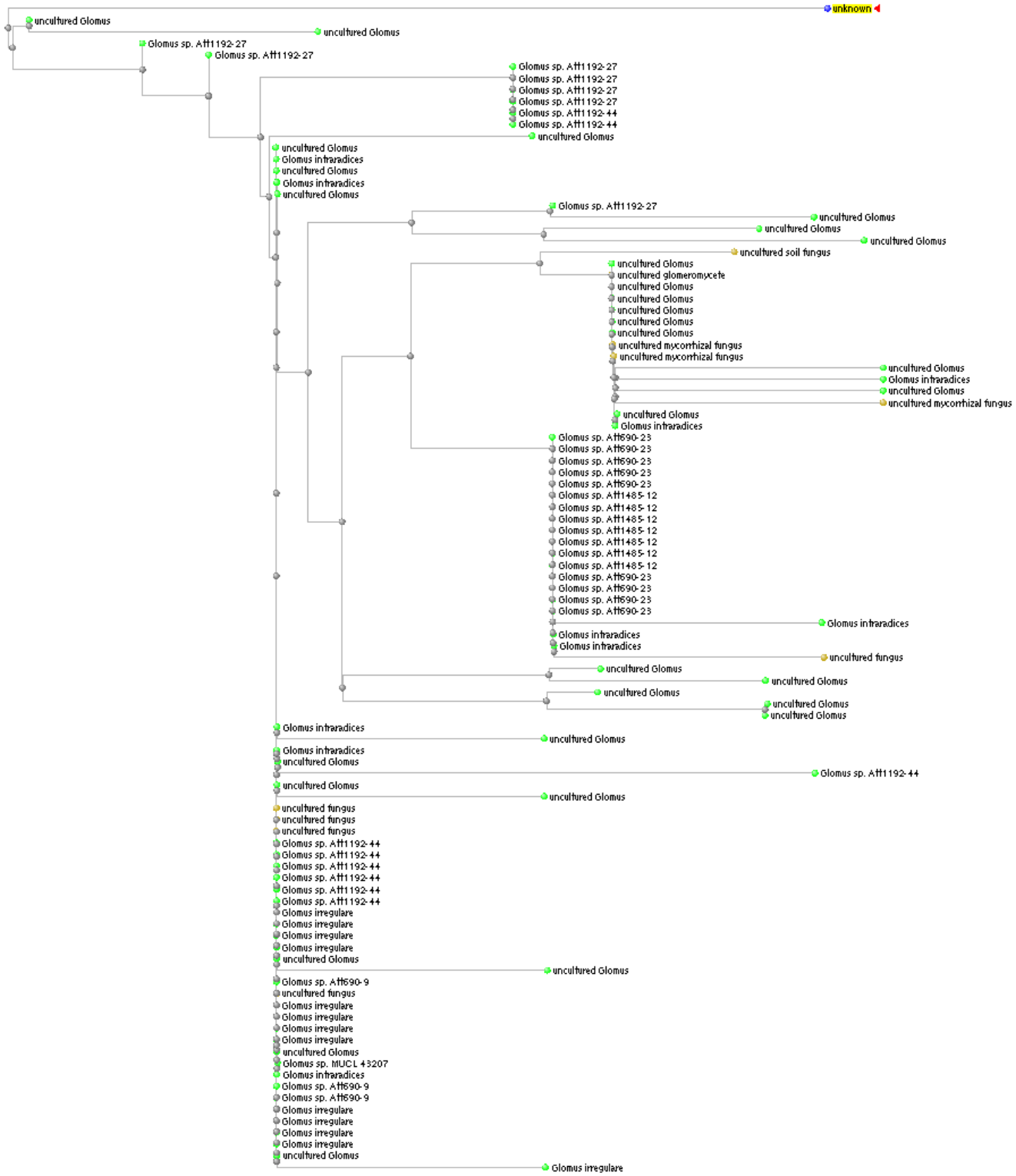
Glomus intraradices

Glom-03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uncultured Glomus
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uncultured Glomus
uncultured Glomus
uncultured mycorrhizal fungus
uncultured mycorrhizal fungus
uncultured Glomus
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uncultured mycorrhizal fungus
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Glomus sp. Att690-23
Glomus sp. Att690-23
Glomus sp. Att690-23
Glomus sp. Att690-23
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Glomus intraradices
Glomus intraradices
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uncultured Glomus
Glomus intraradices
uncultured Glomus
Glomus intraradices
uncultured Glomus
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uncultured Glomus
uncultured Glomus
uncultured fungus
uncultured fungus
uncultured fungus
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Glomus irregulare
Glomus irregulare
Glomus irregulare
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Glomus sp. Att690-9
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Glomus irregulare
Glomus irregulare
Glomus irregulare
uncultured Glomus
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Glomus intraradices
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Glomus sp. Att690-9
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uncultured Glomus
Glomus irregulare

Glom-04


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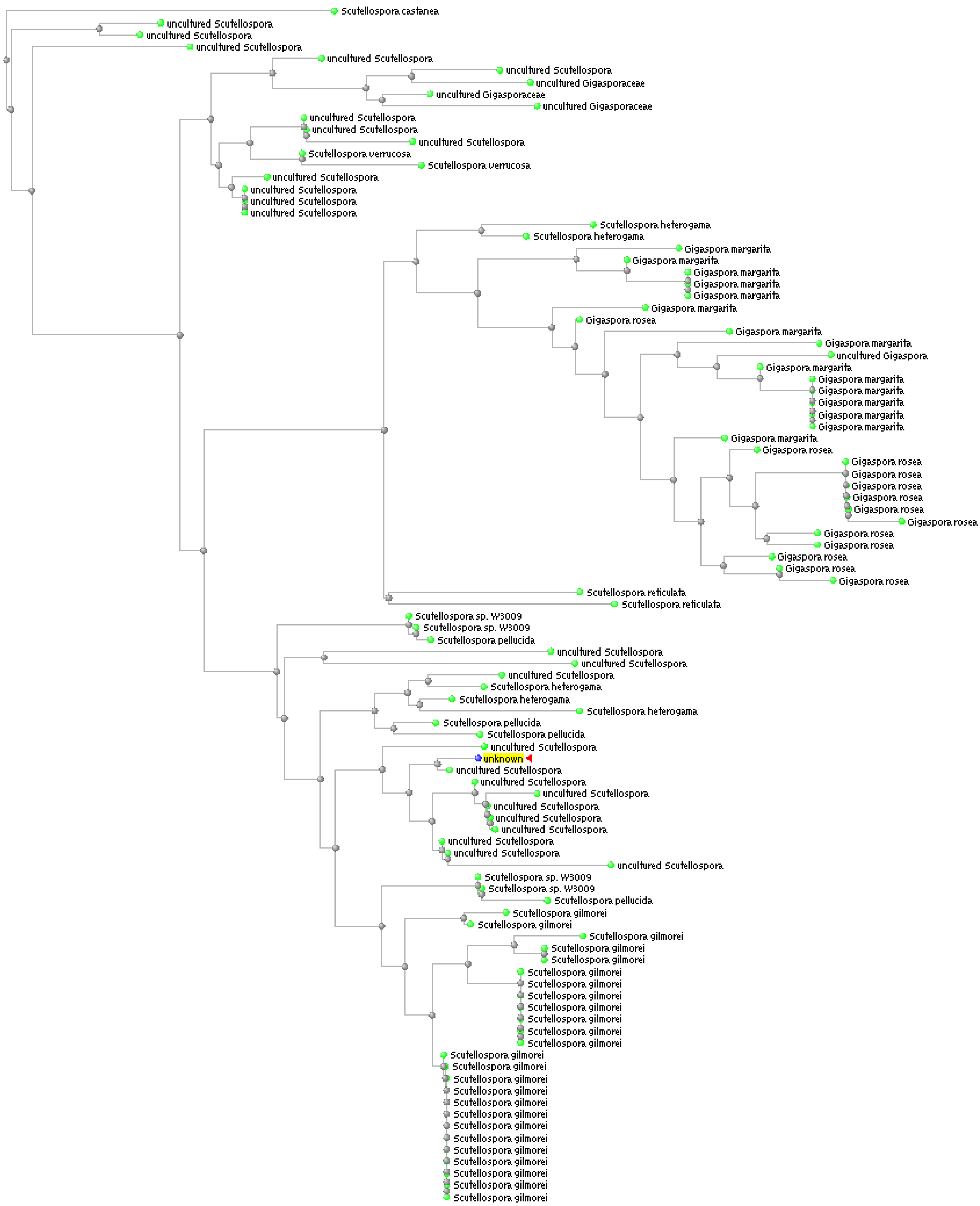
Scut-01



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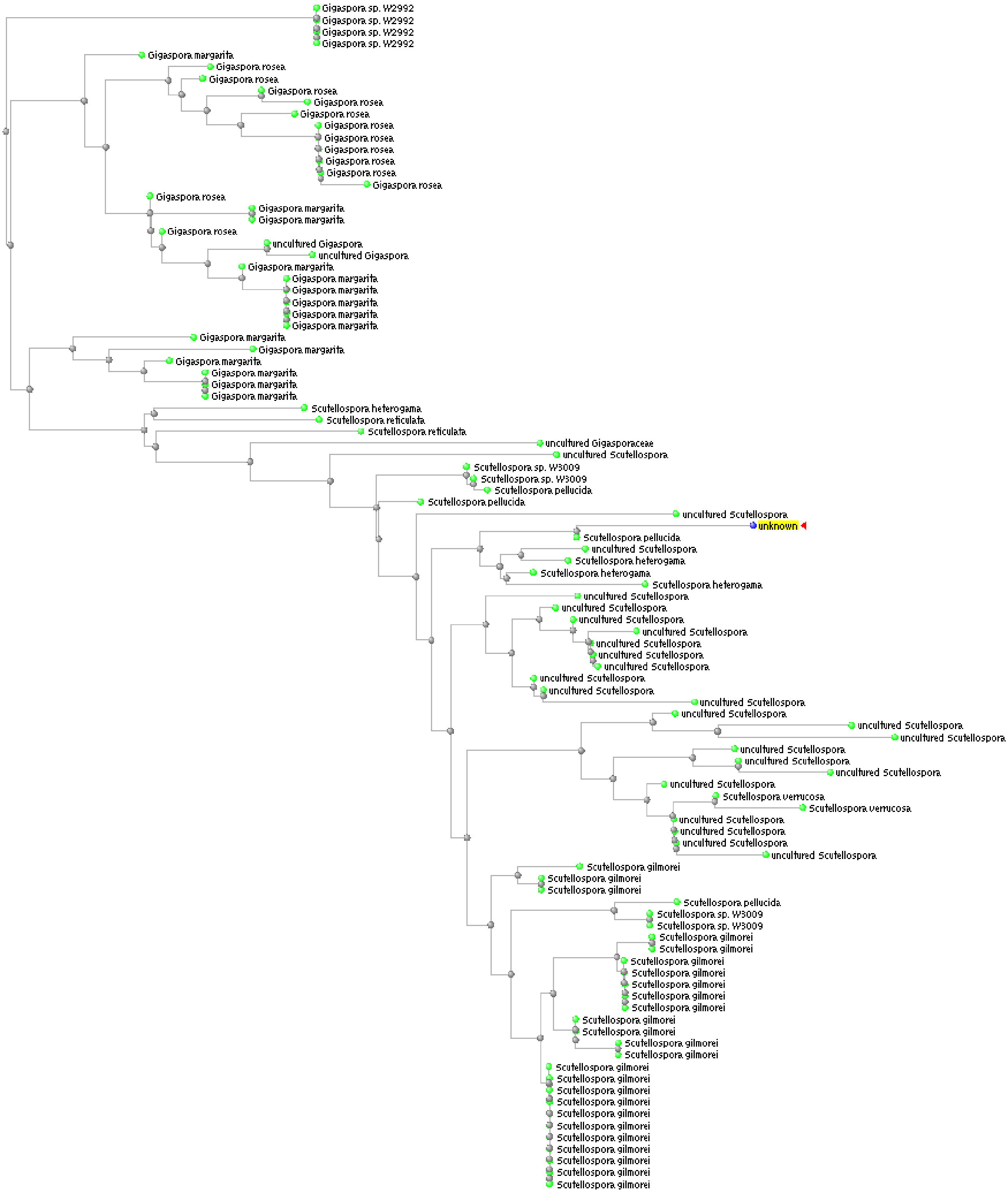
Scut-02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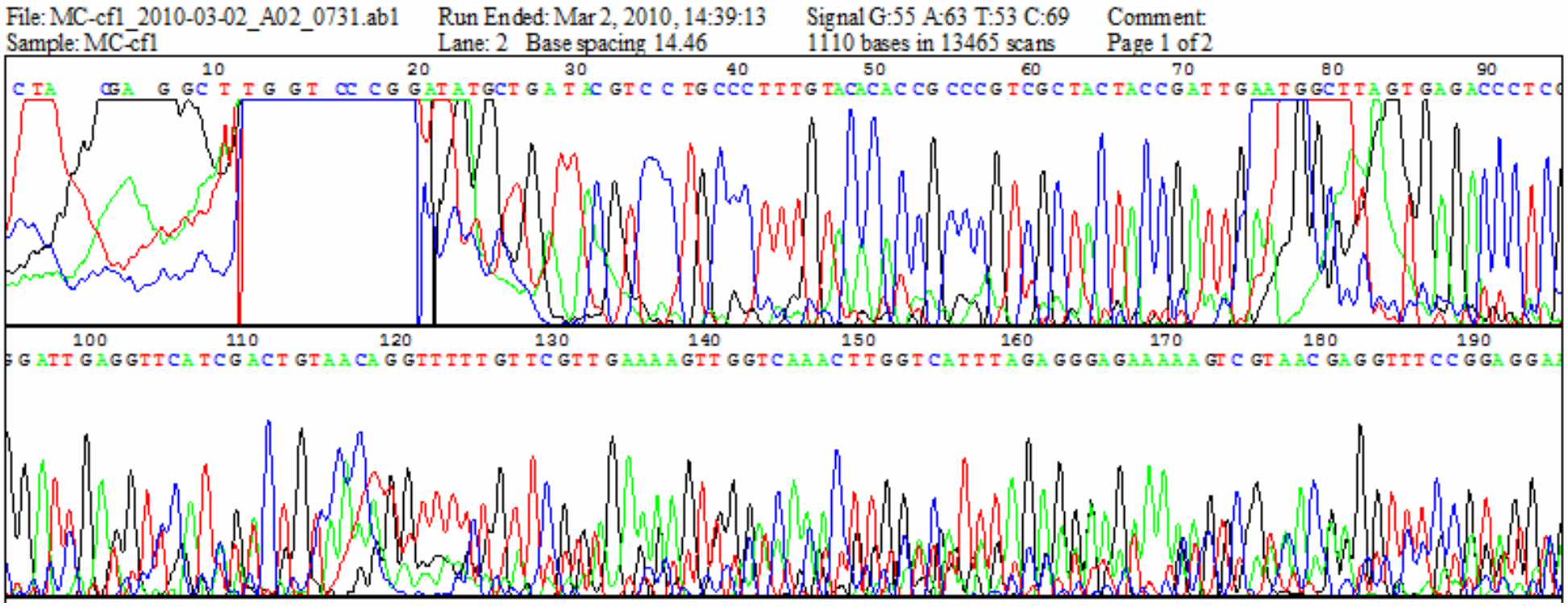
Scutellospora gilmorei

Appendix 4

The electropherograms of each obtained AMF sequence. Each electropherogram only shows the portion of the sequence used for phylogenetic analysis.

Glom-01



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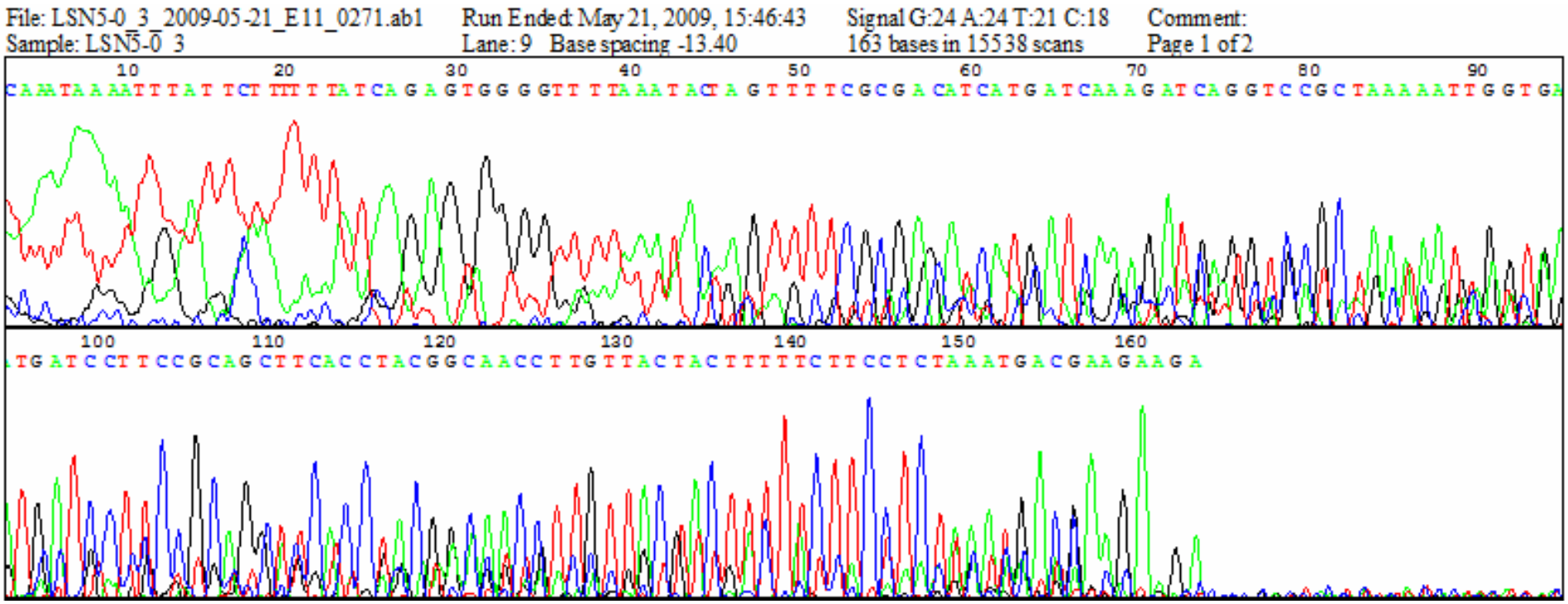
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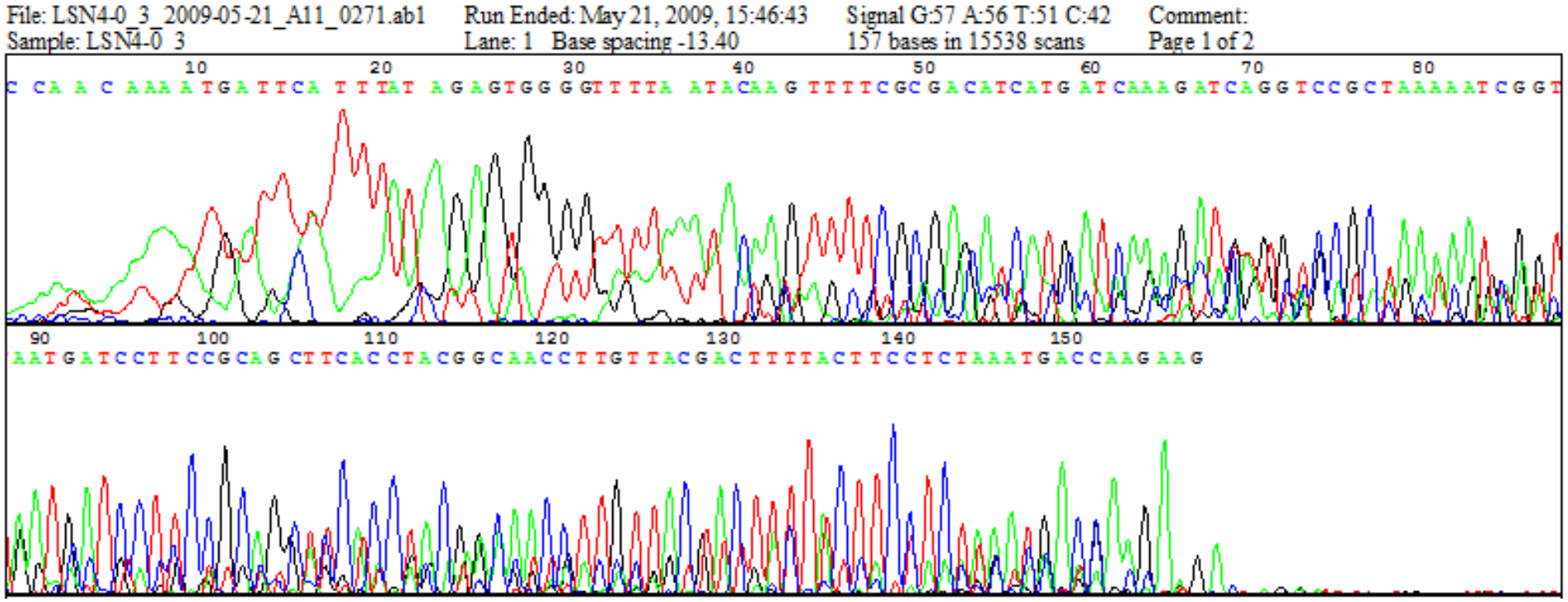
163 bases in 15538 scans
Page 1 of 2

Glom-03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Page 1 of 2
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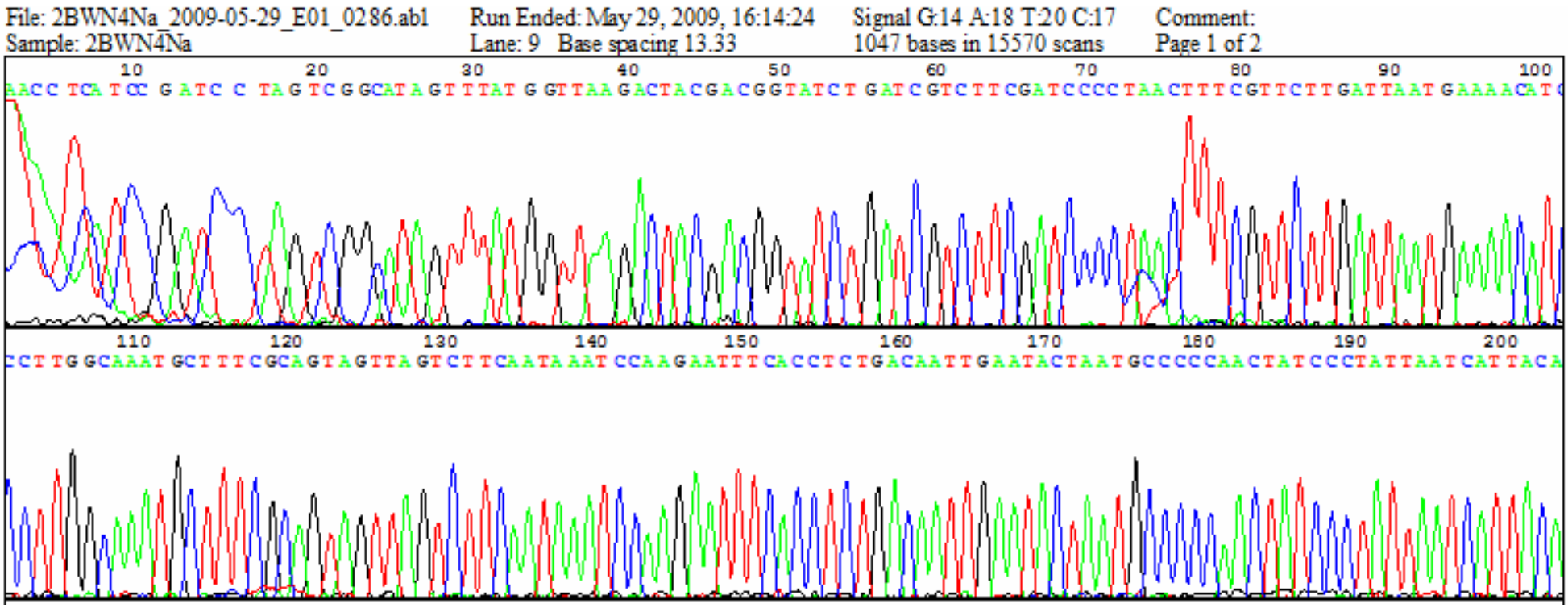
Glom-04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Page 1 of 2
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110 120 130 140 150 160 170 180 190 200
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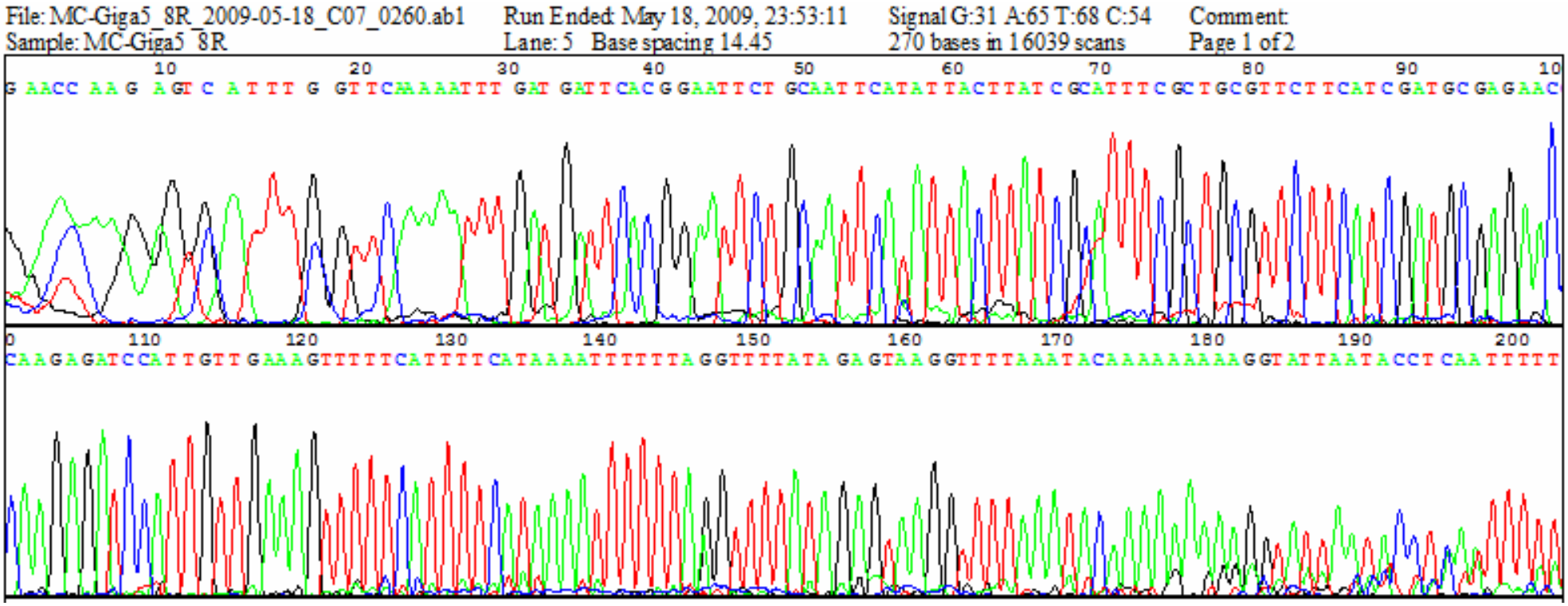
Scut-01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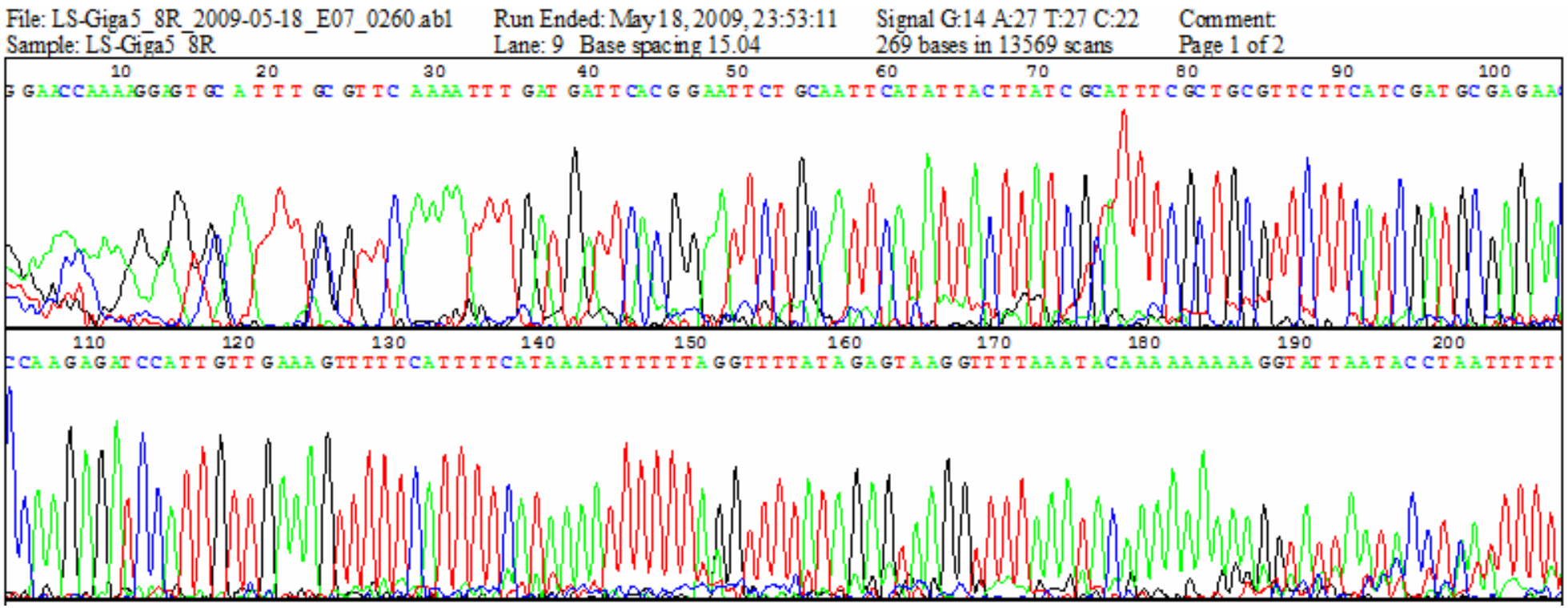
Page 1 of 2

Scut-02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Page 1 of 2

Appendix 5

The difference between nurse plants on the growth and survival of *Podocarpus cunninghamii* seedlings in the Mackenzie Basin, eastern South Island, New Zealand

Introduction

Degradation of ecosystems is occurring worldwide. Based on the model of threshold dynamics, severe degradation can result in an ecosystem passing a threshold level of disturbance and entering an alternative stable state (Suding & Hobbs 2009). These alternative stable states pose a particular problem for ecological restoration because positive feedback mechanisms present within the new system help to maintain the system in the new state, making it difficult to reverse the effects of degradation (Suding *et al.* 2004). In such instances, removal of degrading factors alone (e.g. grazing) without tackling the positive feedbacks (e.g. reductions in soil fertility favouring a different vegetation type) may be insufficient for ecological restoration.

The Mackenzie Basin of eastern South Island, New Zealand, is a semi-arid intermontane environment that receives approximately 600 mm of rainfall per annum. The current vegetation is dominated by native tussock grasses (*Festuca novae-zelandiae* and *Poa colensoi* (both Poaceae) heavily invaded by exotic forbs (*Hieracium pilosella* and *Hieracium praealtum* (Asteraceae)) (Meurk *et al.* 2002). Prior to human arrival much of this landscape was dominated by woody vegetation, with *Podocarpus cunninghamii* (Podocarpaceae) one of the dominant conifer species (McGlone 1989; McGlone 2004). This wooded landscape has all but disappeared as a result of human induced fires and clearance for pastoral development. These anthropogenic disturbances have pushed the original ecosystems across several ecological thresholds (Hobbs & Norton 2004), some of which are irreversible (Walker *et al.* 2009).

Given the sheer extent of deforestation that has occurred in the Mackenzie Basin and similar areas of eastern South Island since the Holocene (Rogers *et al.* 2007), and the conversion of this landscape to grassland, it is likely that changes in the hydrologic and nutrient cycles may also have taken place. The loss of shading previously afforded by tree canopy cover and the increased exposure of the soil to the desiccating and erosive effects of the föhn winds that frequently sweep across the landscape may have led to a reduction in surface soil moisture and nutrient values.

Attempts to restore parts of this landscape back to its former woody vegetation, by planting seedlings of *Podocarpus cunninghamii*, have been met with remarkable failure, despite the use of herbicide, fertiliser and grazing exclusion; exposure to wind and frost have been attributed to the failure (Ledgard 2004). However, studies manipulating the environment, by providing shade and some shelter from frost damage, have found more encouraging results. Payne & Norton (2010) found that beneath shade cloth, herbaceous foliar and surface soil moisture content increased compared to unshaded plots growing in the Mackenzie Basin. In addition, it has been found that in the shelter of felled wilding conifers, the growth of native tussock grasses (*Festuca novae-zelandiae)* and woody shrubs (*Discaria toumatou*) increased compared with paired plots in the open (Paul & Ledgard 2008). These results indicate that the abiotic environment of the deforested high country has altered, and that manipulation to provide woody plants with more shelter may be required for their successful establishment.

A form of shelter that already exists in the high country is provided by extant woody vegetation: remnant patches of grey scrub communities and stands of exotic pines (both managed and wilding). These vegetation communities could potentially be utilised as nurse plants by providing favourable conditions for native tree establishment, by increasing soil moisture values, aiding in the accumulation of soil nutrients, providing protection from herbivores, and by increasing shelter from environmental stressors (Callaway & Walker 1997; Padilla & Pugnaire 2006), such as frost damage and desiccating winds. This study aimed to compare the growth and survival of *Podocarpus cunninghamii* seedlings planted within an exotic pine plantation and a remnant native shrubland within the Mackenzie Basin.

Methods

Two planting environments (pine plantation and native shrubland) were selected from the Cass River Valley that feeds into Lake Tekapo. The pine plantation (*Pinus ponderosa*) was planted as a shelterbelt on an alluvial terrace (approx. 800 m a.s.l.) (43° 51' S, 170° 26' E). The section selected for planting was 0.021 ha in area (8 m × 26 m), had a basal area of 77 $m^2.ha^{-1}$ and a mean top height of 15.41 m; the ground was covered by an exotic grass sward (*Holcus mollis*). The native shrubland community was northeast facing, within a gully above the valley bottom (approx. 900 m a.s.l.) (43° 49' S, 170° 23' E). The shrubland was dominated by *Discaria toumatou*, *Coprosma propinqua* and *Melicytis alpinus*. Seedlings were planted in December 2008. After planting each seedling was tagged and surrounded in hard plastic mesh to prevent damage from lagomorphs. Stock (sheep and cattle) were able to access all paddocks except the pine plantation. The height of each seedling at planting was measured. The survival and height of each seedling was re-assessed over two growth seasons (November 2009 and May 2010).

The relative height growth (RHG) of seedlings within the pine plantation and shrubland after one year were analysed using one-way ANOVA. RHG of seedlings after two years were analysed using a linear mixed effects model, with the error structure of the random effects defined as individual tree within year of measurement. RHG was calculated by ln(final height) – ln(initial height). RHG data were log-transformed to fulfil the assumptions of ANOVA. All seedlings that died were omitted from the analysis. Two *t*-test was also performed on relative growth data, one within the pine plantation and one within the shrubland, to identify if growth rates changed significantly between years one and two.

Seedling survivorship between the pine plantation and shrubland after two years were analysed using a linear mixed effects model on square root arcsine transformed data, with year specified as the random effect.

Results

After one year seedling growth was greatest in the pine plantation, but not significantly greater than growth in the shrubland ($F_{1,23} = 1.83$ $p > 0.1$; Figure A5.1).

After two years, the growth of seedlings within the pine plantation and shrubland remained equivalent ($t_{21,21}$ = 1.13, p > 0.2; Figure A5.1). However, the growth of seedlings within the pine plantation suffered a significant reduction from year one to two (t_{16} = 2.40, p < 0.05), while growth in the shrubland remained unchanged (t_7 = 0.05, p > 0.9).

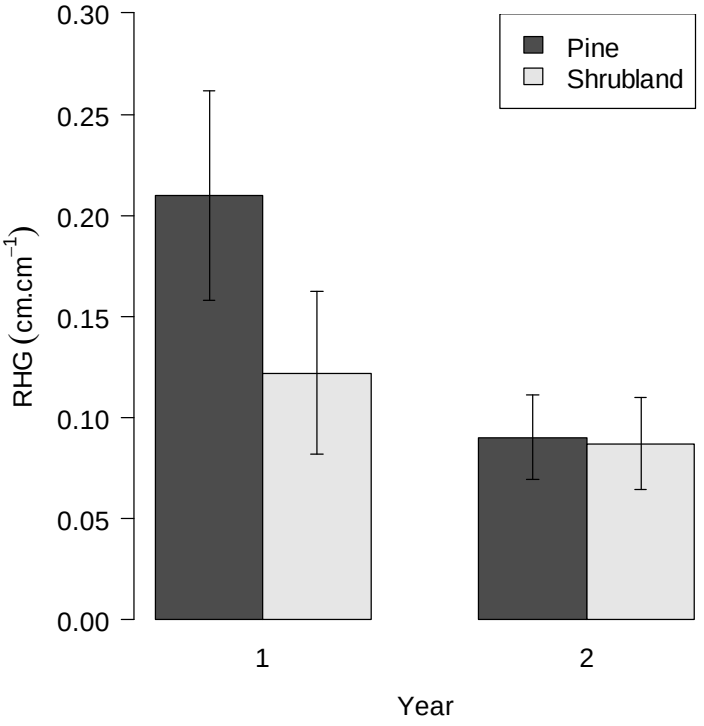

Figure A5.1 – Relative height growth of *Podocarpus cunninghamii* seedlings within the two planting environments over two years.

The survivorship of seedlings was significantly different between the pine plantation and native shrubland ($t_{1,1}$ = 12.97, p < 0.05; Figure A5.2), with survivorship being significantly higher in the pine plantation. The majority of the mortality observed within the shrubland environment appeared attributable to livestock trampling, although several of the deceased did not show any signs of physical damage, suggesting a non-livestock factor was also a cause of mortality. After two years, percent mortality within the shrubland attributable to trampling was 48%, while the remaining deceased, which showed no evidence of trampling damage, was 35% (Figure A5.3).

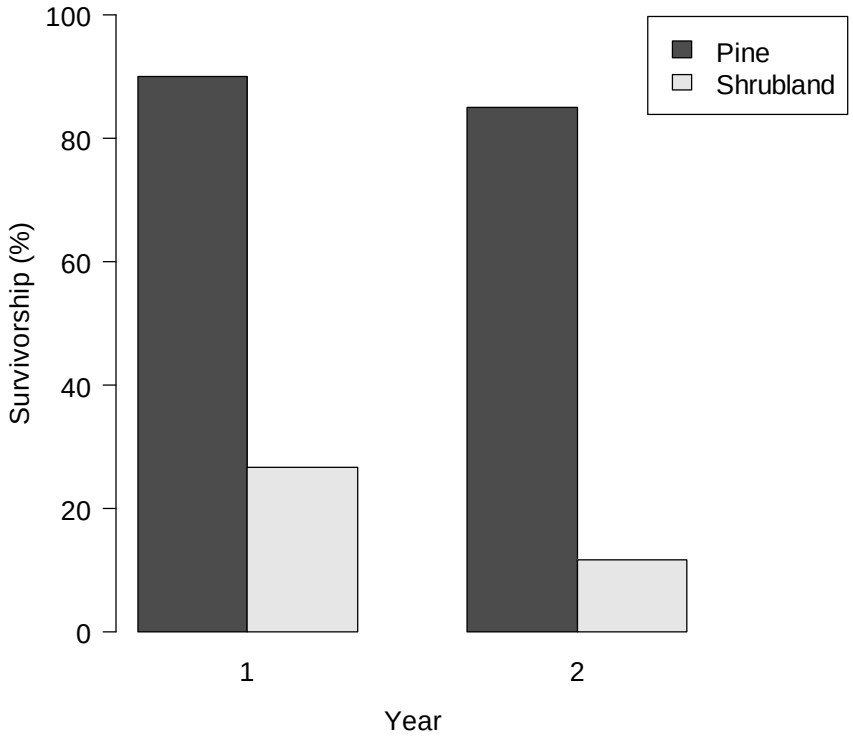

Figure A5.2 – Percentage of *Podocarpus cunninghamii* seedlings surviving within the two planting environments after two years.

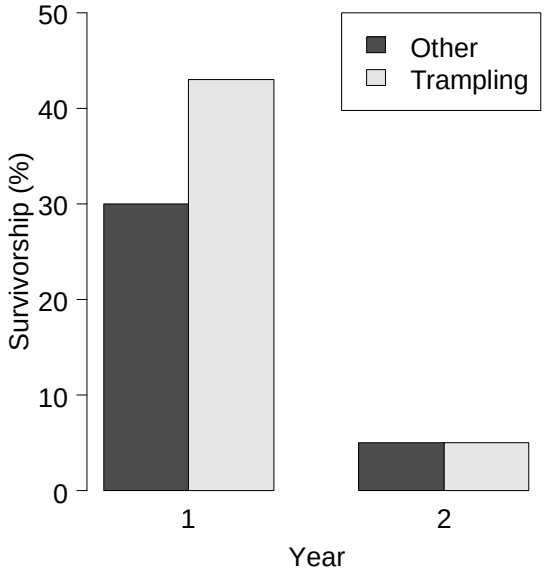

Figure A5.3 – Percentage *Podocarpus cunninghamii* seedling mortality within native shrubland attributable to trampling.

Discussion

The results of this study show that, after two growing seasons, the growth rate of *Podocarpus cunninghamii* seedlings planted within a pine plantation was not significantly different from seedlings planted within a native shrubland. This suggests that both the exotic pine plantation and native shrubland environments have equivalent effects on *Podocarpus cunninghamii* seedling growth.

In terms of seedling survivorship the native shrubland performed poorly. After one year seedling mortality was 73% (Figure A5.3). This suggests that native shrublands provide poor nurse cover. It should be noted that the majority of this mortality was attributable to livestock trampling. As such, despite the shrublands being thorny, due to *Discaria toumatou*, they were unable to provide protection from large domestic livestock (cattle). However, trampling aside, seedling mortality within the shrubland in year one was 30%. This rate of mortality after one year is still much greater than that recorded for seedlings planted in the pine plantation (10%).

Seedling survivorship within the pine plantation, after two years, was high (85%), and suggests that *Pinus ponderosa* pine plantations may be suitable for providing nurse cover to *Podocarpus cunninghamii* seedlings. This result contrasts markedly with that of Ledgard (2004), who reported 100% mortality of *Podocarpus cunninghamii* seedlings after four years beneath a nurse cover of *Pinus radiata*, within the Mackenzie Basin. This high level of mortality was attributed to a drought event in the summer of 1998/99, which caused greater water stress for seedlings planted beneath a nurse crop compared to those planted in open grassland, where survivorship after four years was 39%; no drought was recorded in the two years of the current study. Another possible reason for the difference in *Podocarpus cunninghamii* seedling survival between the two pine plantations was that the one used in the current study was established as a shelterbelt, meaning it was narrow (8 m wide). Resultantly, although the seedlings were beneath a closed canopy, they still received plentiful 'side-light'. This was not the case for the seedlings in the Ledgard (2004) trial, where they were planted within a large plantation, and excessive shading may have increased mortality.

The results of this study showed that native shrubland was also unable to provide protection to seedlings from livestock. The best way to achieve this appears to be by erecting stock proof fencing, as was done for seedlings planted in the pine plantation. Future work should reinvestigate the potential nurse effects of native shrublands once stock proof fencing has been erected. In addition, this study compared differences in seedling growth and survival between different types of nurse plants, but not against seedlings planted without cover; this was done solely due to limitations in available *Podocarpus cunninghamii* seedlings of high country provenance. As such it is not possible to say whether the presence of nurse cover provided any benefit to *Podocarpus cunninghamii* seedlings compared with seedlings planted without cover. Future studies should make such a comparison. Finally, the effect of nurse plants may require more than one or two growth seasons to take effect, and as such this trial should continue to be monitored over the coming years.

References

Callaway, R.M. & Walker, L.R. (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology,* **78,** 1958-1965.

Hobbs, R.J. & Norton, D.A. (2004) Ecological filters, thresholds, and gradients in resistance to ecosystem reassembly. *Assembly Rules and Restoration Ecology: Bridging the Gap between Theory and Practice* (eds V.M. Temperton, R.J. Hobbs, T. Nuttle & S. Halle), pp. 72-95. Island Press, Washington.

Ledgard, N.J. (2004) Establishing native species in the high country. *High Country Forestry*, pp. 2-4. Forest Research, Christchurch.

McGlone, M.S. (1989) The Polynesian settlement of New Zealand in relation to environmental and biotic changes. *New Zealand Journal of Ecology,* **12,** 115-129.

McGlone, M.S. (2004) *Vegetation history of the South Island high country*. Landcare Research, Lincoln.

Meurk, C.D., Walker, S., Gibson, R.S. & Espie, P. (2002) Changes in vegetation states in grazed and ungrazed Mackenzie Basin grasslands, New Zealand, 1990-2000. *New Zealand Journal of Ecology,* **26,** 95-106.

Padilla, F.M. & Pugnaire, F. (2006) The role of nurse plants in the restoration of degraded environments. *Frontiers in Ecology and the Environment,* **4,** 196-202.

Paul, T.S.H. & Ledgard, N.J. (2008) Effect of felled wilding pines on plant growth in high country grasslands. *New Zealand Plant Protection,* **61,** 105-110.

Payne, T. & Norton, D.A. (2010) Thresholds to restoration in degraded tussock grasslands: the influence of shade on soil moisture. *Unpublished manuscript*, pp. 1-15. School of Forestry, University of Canterbury, Christchurch.

Rogers, G.M., Walker, S., Basher, L.M. & Lee, W.G. (2007) Frequency and impact of Holocene fire in eastern South Island, New Zealand. *New Zealand Journal of Ecology,* **31,** 129-142.

Suding, K.N., Gross, K.L. & Houseman, G.R. (2004) Alternative states and positive feedbacks in restoration ecology. *Trends in Ecology & Evolution,* **19,** 46-53.

Suding, K.N. & Hobbs, R.J. (2009) Models of ecosystem dynamics as frameworks for restoration ecology. *New Models for Ecosystem Dynamics and Restoration* (eds R.J. Hobbs & K.N. Suding). Island Press, Washington, DC.

Walker, S., Cieraad, E., Monks, A., Burrows, L., Wood, J., Price, R., Rogers, G. & Lee, B. (2009) Long-term dynamics and rehabilitation of woody ecosystems in dryland South Island, New Zealand. *New Models for Ecosystem Dynamics and Restoration* (eds R.J. Hobbs & K.N. Suding), pp. 99-111. Island Press, Washington, DC.